

EICOSANOID REGULATION OF HEMATOPOIETIC STEM AND
PROGENITOR CELL FUNCTION

Jonathan G. Hoggatt

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Doctor of Philosophy
in the Department of Microbiology and Immunology,
Indiana University

May 2010

Accepted by the Faculty of Indiana University, in partial
fulfillment of the requirements for the degree of Doctor of Philosophy.

Louis M. Pelus Ph.D., Chair

Hal E. Broxmeyer Ph.D.

Doctoral Committee

D. Wade Clapp M.D.

April 5, 2010

Edward Srour Ph.D.

Acknowledgements

I would first like to thank my mentor, Dr. Louis M. Pelus, who has extended an incredible amount of trust in me and created an environment with a remarkable degree of freedom. I was given a true "behind the scenes" education and had access to his support and ideas days, nights and weekends, of which I am truly grateful.

I thank my fellow Pelus lab members, both past and present, Dr. Seiji Fukuda, Dr. Janardhan Sampath, Dr. Javier Rivera Guzman, Lissa Smith and Jennifer Speth, who have always been willing to lend a helpful hand. In particular, I would like to thank Dr. Pratibha Singh, who without her support, friendship and guidance, the work presented herein would not be possible.

Thanks go to my committee members, Dr. Hal E. Broxmeyer, Dr. D. Wade Clapp and Dr. Edward Srouf, who have allowed me to poke my head in their offices countless times and ask for their thoughts and suggestions regarding this dissertation work. I would also like to thank Dr. Christie Orschell for her assistance in the radiation studies and a similar open door policy.

Finally, I thank my wife, Dr. Amber Hoggatt, who I was very fortunate to find in my life. Not many wives would be willing to work 90 to 100 hour weeks in the lab through an entire summer, but much of this work would not have been feasible without your help. Co-publishing a manuscript with you will always be a fond memory, and I thank you for your continued support.

Abstract

Jonathan G. Hoggatt

EICOSANOID REGULATION OF HEMATOPOIETIC STEM AND PROGENITOR CELL FUNCTION

Adult hematopoietic stem cells (HSC) are routinely used to reconstitute hematopoiesis after myeloablation; however, transplantation efficacy and multilineage reconstitution can be limited by inadequate HSC number, or poor homing, engraftment or self-renewal. We have demonstrated that mouse and human HSC express prostaglandin E₂ (PGE₂) receptors, and that short-term *ex vivo* exposure of HSC to PGE₂ enhances their homing, survival and proliferation, resulting in increased long-term repopulating cell and competitive repopulating unit (CRU) frequency. HSC pulsed with PGE₂ are more competitive, as determined by head-to-head comparison in a competitive transplantation model. Enhanced HSC frequency and competitive advantage is stable and maintained upon multiple serial transplantations, with full multi-lineage reconstitution. PGE₂ increases HSC CXCR4 mRNA and surface expression and enhances their migration to SDF-1 α *in vitro* and homing to bone marrow *in vivo* and stimulates HSC entry into and progression through cell cycle. In addition, PGE₂ enhances HSC survival, associated with an increase in Survivin mRNA and protein expression and reduction in intracellular active caspase-3. While PGE₂ pulse of HSC promotes HSC self-renewal, blockade of PGE₂ biosynthesis with non-

steroidal anti-inflammatory drugs (NSAIDs) results in expansion of bone marrow hematopoietic progenitor cells (HPC). We co-administered NSAIDs along with the mobilizing agent granulocyte-colony stimulating factor (G-CSF) and evaluations of limiting dilution transplants, assays monitoring neutrophil and platelet recoveries, and secondary transplantations, clearly indicate that NSAIDs facilitate mobilization of a hematopoietic graft with superior functional activity compared to the graft mobilized by G-CSF alone. Enhanced mobilization has also been confirmed in baboons mobilized with G-CSF and a NSAID. Increases in mobilization are the result of a reduction of signaling through the PGE₂ receptor EP4, which results in marrow expansion and reduction in the osteoblastic HSC niche. We also identify a new role for cannabinoids, an eicosanoid with opposing functions to PGE₂, in hematopoietic mobilization. Additionally, we demonstrate increased survival in lethally irradiated mice treated with PGE₂, NSAIDs, or the hypoxia mimetic cobalt chloride. Our results define novel mechanisms of action whereby eicosanoids regulate HSC and HPC function, and characterize novel translational strategies for hematopoietic therapies.

Louis M. Pelus Ph.D., Chair

Table of Contents

| | |
|--|----------|
| List of Tables | xi |
| List of Figures | xii |
| List of Abbreviations | xv |
| | |
| Chapter 1. Introduction | 1 |
| Hematopoiesis – Stem and progenitor cells and colony assays | 1 |
| Hematopoiesis – Competitive transplantation assays | 5 |
| Hematopoiesis – Immunophenotypic characterization of HSC and HPC | 8 |
| Hematopoiesis – The “hematopoietic tree” | 11 |
| Hematopoiesis – The hematopoietic “niche” | 16 |
| Hematopoietic Trafficking – Homing and mobilization | 19 |
| Eicosanoid Biosynthesis | 24 |
| Eicosanoid Receptors and Signaling | 32 |
| Prostaglandins and Hematopoiesis – Stimulatory and inhibitory functions | 40 |
| The Plot Thickens – Leukotrienes and endocannabinoids | 43 |
| The Yin and Yang of Eicosanoid Regulation | 45 |

| | |
|---|-----------|
| Chapter 2. Prostaglandin E₂ Enhances Hematopoietic Stem Cell Homing, Survival and Proliferation | 49 |
| Introduction | 49 |
| Materials and Methods | 51 |
| Mice and human cord blood | 51 |
| Flow cytometry | 51 |
| dmPGE ₂ pulse-exposure | 52 |
| Limiting dilution competitive and non-competitive transplantation | 52 |
| Long-term competitiveness assay | 53 |
| Analysis of HSC homing | 54 |
| Expression of EP receptors, CXCR4 and Survivin | 55 |
| Migration assays | 56 |
| Cell cycle analysis | 56 |
| Apoptosis assays | 57 |
| Quantitative-RT-PCR | 57 |
| Statistical analysis | 58 |
| QRT-PCR primer sequences | 59 |
| Results | 60 |
| PGE ₂ increases LTR-HSC frequency and engraftment | 60 |
| Lack of inherent enhanced competitiveness of dmPGE ₂ pulsed grafts in secondary transplants | 70 |
| Murine and human HSC and HPC express EP receptors | 74 |
| PGE ₂ increases HSC homing efficiency | 79 |

| | |
|---|---------|
| PGE ₂ increases HSC CXCR4 and chemotaxis to SDF-1 α | 82 |
| PGE ₂ decreases HSC apoptosis and increases Survivin | 94 |
| PGE ₂ increases HSC proliferation | 97 |
| Discussion | 103 |
| Chapter 3. Modulation of Eicosanoid Signaling to Enhance Hematopoietic Expansion and Mobilization | 110 |
| Introduction | 110 |
| Materials and Methods | 113 |
| Mice and baboons | 113 |
| Peripheral blood and bone marrow acquisition and processing | 114 |
| Colony assays | 115 |
| Flow cytometry | 115 |
| Peripheral blood mobilization | 116 |
| Limiting dilution competitive transplantation | 117 |
| Recovery assay | 118 |
| Results | 119 |
| NSAID treatment results in increased HPC | 119 |
| NSAID treatment enhances mobilization of HSC and HPC | 122 |
| Enhanced mobilization requires inhibition of COX1 and COX2 | 135 |
| NSAID co-administration with G-CSF facilitates mobilization of a superior hematopoietic graft with enhanced recovery and long-term repopulation | 139 |

| | |
|---|-----|
| NSAID facilitated grafts enhance hematopoietic recovery | 149 |
| NSAIDs facilitate mobilization in non-human primates | 150 |
| Enhancement in mobilization is mediated through a reduction in EP4 receptor signaling | 159 |
| Reduced bone marrow osteoblasts and increased myelopoiesis in NSAID treated mice | 165 |
| Cannabinoid receptor agonism mobilizes HPC | 168 |
| Discussion | 175 |
| Chapter 4. Recovery From Hematopoietic Injury by Modulating PGE₂ Signaling or HIF-1α Post-Irradiation | 184 |
| Introduction | 184 |
| Materials and Methods | 186 |
| Mice | 186 |
| Radiation | 187 |
| Post-irradiation treatment | 187 |
| Morbidity and mortality monitoring | 188 |
| Colony assays | 188 |
| Results | 189 |
| PGE ₂ treatment increases survival post-irradiation | 189 |
| Radiomitigation with delayed NSAID administration | 193 |
| Cobalt Chloride administration increases survival post-irradiation | 194 |
| Discussion | 196 |

| | |
|--|-----|
| Chapter 5. Future Directions | 201 |
| <i>Ex vivo</i> pulse with PGE ₂ | 201 |
| Additional agents to increase homing | 204 |
| Mobilization of bone marrow-derived progenitor cells | 205 |
| Mobilization with other agents | 208 |
| Eicosanoid regulation to improve gene therapy | 209 |
| Clinical trials and patent applications | 213 |
| HSC and HPC fate decisions directed by eicosanoids | 215 |
| References | 216 |
| Curriculum Vitae | |

List of Tables

Chapter 1

| | | |
|----------------|---|----|
| Table 1 | Opposing roles of cannabinoids, leukotrienes and prostaglandins | 47 |
|----------------|---|----|

Chapter 2

| | | |
|----------------|--|-----|
| Table 2 | QRT-PCR primer sequences | 59 |
| Table 3 | PGE ₂ pulsed grafts have increased LTRC | 64 |
| Table 4 | Effects of short term <i>in vitro</i> exposure of SLAM SKL cells to dmPGE ₂ on cell cycle | 101 |

Chapter 3

| | | |
|----------------|--|-----|
| Table 5 | Blockade of PGE ₂ inhibition of CFU-M by EP4 antagonism | 163 |
|----------------|--|-----|

List of Figures

Chapter 1

| | | |
|-----------------|---|----|
| Figure 1 | The hematopoietic tree | 14 |
| Figure 2 | Schematic of eicosanoid biosynthesis | 30 |
| Figure 3 | Schematic representation of EP receptor signaling | 34 |
| Figure 4 | Schematic representation of leukotriene and endocannabinoid signaling | 38 |

Chapter 2

| | | |
|------------------|---|----|
| Figure 5 | PGE ₂ enhances hematopoietic stem cell engraftment | 61 |
| Figure 6 | Full multi-lineage reconstitution after transplantation with PGE ₂ pulsed grafts | 66 |
| Figure 7 | PGE ₂ pulsed grafts maintain repopulating ability through serial transplantations | 68 |
| Figure 8 | dmPGE ₂ pulsed HSC do not have inherent competitive advantage in secondary transplants | 72 |
| Figure 9 | EP receptors are expressed on murine and human HSC and HPC | 75 |
| Figure 10 | Amplification plots of mRNA for PGE ₂ receptors | 77 |
| Figure 11 | PGE ₂ increases homing efficiency of HSC | 80 |
| Figure 12 | Competitive head-to-head increase in HSC homing | 83 |
| Figure 13 | PGE ₂ increases homing efficiency of UCB CD34 ⁺ cells in NSG mice | 85 |
| Figure 14 | CXCR4 receptor expression is increased on murine and human HSC and HPC after dmPGE ₂ treatment | 87 |
| Figure 15 | Increase in HSC and HPC migration to SDF-1 α after dmPGE ₂ treatment | 90 |

| | | |
|------------------|---|----|
| Figure 16 | PGE ₂ enhancement of migration and homing of HSC and HPC is inhibited by AMD3100 | 92 |
| Figure 17 | PGE ₂ decreases apoptosis, increases Survivin expression and decreases active caspase-3 in HSC | 95 |
| Figure 18 | PGE ₂ increases the proliferation of SKL cells | 99 |

Chapter 3

| | | |
|------------------|--|-----|
| Figure 19 | Dual COX inhibitory NSAIDs expand HPC within BM | 120 |
| Figure 20 | Indomethacin enhances mobilization of CFU-GM | 124 |
| Figure 21 | NSAIDs increase mobilization of functionally and phenotypically defined HSC and HPC | 126 |
| Figure 22 | Representative CFU-GM colonies from G-CSF and G-CSF plus NSAID mobilized mice. | 128 |
| Figure 23 | NSAIDs synergistically increase mobilization with AMD3100 | 131 |
| Figure 24 | Enhancement in mobilization by NSAIDs is independent of mobilizing agent mechanism | 133 |
| Figure 25 | Enhancement in mobilization requires inhibition of COX1 and COX2 | 137 |
| Figure 26 | Non-staggered administration of G-CSF and NSAID results in no increase in CRU in the mobilized graft | 141 |
| Figure 27 | Staggered administration of NSAID with G-CSF restores CXCR4 receptor expression on HSC | 143 |
| Figure 28 | Staggered administration of NSAID with G-CSF enhances mobilized graft repopulating ability | 145 |

| | | |
|-----------------------------|--|-----|
| Figure 29 | Staggered administration of NSAID with G-CSF enhances mobilized graft LTRC frequency | 147 |
| Figure 30 | NSAID co-administration enhances neutrophil and platelet recovery capacity of G-CSF mobilized grafts | 151 |
| Figure 31 | NSAID facilitated grafts retain long-term repopulating ability | 153 |
| Figure 32 | Enhanced mobilization in baboons with co-administration of Meloxicam with G-CSF | 155 |
| Figure 33 | Meloxicam administration increases CD34 ⁺ cells and CFU-GM in peripheral blood in baboons | 157 |
| Figure 34 | Enhanced mobilization mediated through a reduction in EP4 receptor signaling | 161 |
| Figure 35 | NSAIDs increase myelopoiesis and reduce osteoblasts in the bones of treated mice | 166 |
| Figure 36 | HSC and HPC express CB receptors and CB agonism decreases CXCR4 and VLA-4 | 169 |
| Figure 37 | Cannabinoid agonism through the CB ₂ receptor rapidly and synergistically mobilizes HPC | 173 |
| <u>Chapter 4</u> | | |
| Figure 38 | Radiomitigation by treatment with dmPGE ₂ , Meloxicam, or CoCl ₂ | 191 |
| <u>Chapter 5</u> | | |
| Figure 39 | Specific agonism of EP4 increases CXCR4 receptor expression on CD34 ⁺ UCB cells | 202 |
| Figure 40 | LTB4 antagonism expands CD34 ⁺ UCB cells | 211 |

List of Abbreviations

| | |
|-----------------|--|
| 2-AG | 2-Arachidonoyl glycerol |
| 5-FU | 5-fluorouracil |
| AA | Arachidonic acid |
| APC | Allophycocyanin |
| BFU-E | Burst forming unit-erythrocyte |
| BM | Bone Marrow |
| BrdU | Bromodeoxyuridine |
| BSA | Bovine Serum Albumin |
| CAFC | Cobblestone area-forming cell |
| cAMP | cyclic-Adenosine monophosphate |
| CaR | Calcium sensing receptor |
| CB | Cannabinoid |
| CD | Cluster domain |
| CDC | Centers for Disease Control and Prevention |
| CDP | Common dendritic progenitor cell |
| CFC | Colony forming cell |
| CFU-BL | Blast colony-forming cell |
| CFU-E | Colony-forming unit-erythroid |
| CFU-G | Colony-forming unit-granulocyte |
| CFU-GEMM | Colony-forming unit-granulocyte erythroid macrophage megakaryocyte |
| CFU-GM | Colony-forming unit-granulocyte, macrophage |

| | |
|-------------------------|--|
| CFU-M | Colony-forming unit-monocyte/macrophage |
| CFU-Mk(Meg) | Colony-forming unit-megakaryocyte |
| CFU-S | Colony-forming unit-spleen |
| CFSE | 5-(and -6)-carboxyfluorescein diacetate succinimidyl ester |
| c-kit | Stem cell factor receptor (CD117) |
| CLP | Common lymphoid progenitor cell |
| CMEP | Common myeloerythroid progenitor cell |
| CMLP | Common myelolymphoid progenitor cell |
| CMP | Common myeloid progenitor cell |
| COX | Cyclooxygenase enzyme |
| cPLA₂ | Cytosolic phospholipase A ₂ |
| CRU | Competitive repopulating unit |
| CXCR4 | CXC chemokine receptor-4 |
| Cy7 | Cyanine dye 7 |
| db-cAMP | Dibutyl cyclic-Adenosine monophosphate |
| DNA | Deoxyribonucleic acid |
| DTPA | Diethylenetriaminedipentaacetate |
| EDTA | Ethylenediaminetetraacetic acid |
| ECFC | Endothelial colony forming cell |
| ERK | Extracellular signal-related kinase |
| EP | Prostaglandin E ₂ receptor |
| EPC | Endothelial progenitor cell |
| EPO | Erythropoietin |
| ETOH | Ethanol |

| | |
|---------------------------------|--|
| FAAH | Fatty acid amide hydrolase |
| FACS | Fluorescence activated cell sorting/sorter |
| FDA | Food and Drug Administration |
| FITC | Fluorescein isothiocyanate |
| Flt3 | <i>fms</i> -related tyrosine kinase-3 |
| FSC | Forward scatter |
| G-CSF | Granulocyte colony-stimulating factor |
| G-CSFR | Granulocyte colony-stimulating factor receptor |
| GI | Gastrointestinal |
| GMP | Granulocyte-monocyte progenitor cell |
| GPCR | G-protein coupled receptor |
| GSK-3 | Glycogen synthase kinase-3 |
| GVHD | Graft-versus-host disease |
| Gy | Grey (measure of radiation exposure) (1Gy=100 rads) |
| H&E | Hematoxylin and eosin |
| HIF-1α | Hypoxia inducible factor-1 α |
| HI-FBS | Heat-inactivated fetal bovine serum |
| HLA-DR | Histocompatibility lymphocyte antigen-DR |
| HPC | Hematopoietic progenitor cell |
| HS-ARS | Hematopoietic syndrome of the acute radiation syndrome |
| HSC | Hematopoietic stem cell |
| IACUC | Institutional animal care and use committee |
| IL-1 | Interleukin-1 |
| IL-2 | Interleukin-2 |

| | |
|--------------------------|---|
| IRB | Institutional review board |
| IP | Intraperitoneal |
| IT-HSC | Intermediate-term hematopoietic stem cell |
| LDMC | Low density mononuclear cells |
| Lin^{neg} | Lineage negative cells |
| LOX | Lipoxygenase enzyme |
| LPAM-1 | Lymphocyte Peyer's patch adhesion molecule-1 |
| LPS | Lipopolysaccharides |
| LT | Leukotriene |
| LTC-IC | Long-term culture-initiating cell |
| LT-HSC | Long-term hematopoietic stem cell |
| LTRC | Long-term repopulating cell |
| MAPK | Mitogen-activated protein kinase |
| M-CSF | Macrophage colony stimulating factor |
| MEM | Modified essential medium |
| MEP | Megakaryocyte-erythrocyte progenitor cell |
| MFI | Mean fluorescence intensity |
| MMP-9 | Matrix metalloproteinase-9 |
| MPP | Multipotent progenitor cell |
| NOD/SCID | Non-obese diabetic severe combined immunodeficient mice |
| NSAID | Non-steroidal anti-inflammatory drug |
| NSG | NOD/SCID IL-2 receptor gamma null mice |
| OPN | Osteopontin |
| PB | Peripheral blood |

| | |
|---------------------------------|---|
| PBS | Phosphate buffered saline |
| PBSC | Peripheral blood stem cell |
| PE | Phycoerythrin |
| PI3K | Phosphoinositide-3 kinase |
| PGE₂ | Prostaglandin E ₂ |
| PGs | Prostaglandins |
| PKA | Protein kinase A |
| PKC | Protein kinase C |
| PLA₂ | Phospholipase A ₂ |
| PLC | Phospholipase C |
| PTH | Parathyroid hormone |
| QRT-PCR | Quantitative reverse transcriptase polymerase chain reaction |
| RNA | Ribonucleic acid |
| ROS | Reactive oxygen species |
| RU | Repopulating unit |
| Sca-1 | Stem cell antigen-1 |
| SDF-1α | Stromal cell-derived factor-1 alpha |
| SEM | Standard error of the mean |
| SKL | Sca-1 ⁺ , c-kit ⁺ , Lin ^{neg} cells |
| SLAM | Signaling lymphocyte activation marker |
| SNS | Strategic National Stockpile |
| ST-HSC | Short-term hematopoietic stem cell |
| Tie2 | Angiopoietin receptor tyrosine kinase with immunoglobulin-like and EGF-like domains-2 |

| | |
|---------------|------------------------------------|
| TPO | Thrombopoietin |
| UCB | Umbilical cord blood |
| VCAM-1 | Vascular cell adhesion molecule-1 |
| VEGF | Vascular endothelial growth factor |
| VLA-4 | Very late antigen-4 |
| VLA-5 | Very late antigen-5 |

Chapter 1. Introduction

Higher organisms have the remarkable capacity to produce and maintain adequate numbers of blood cells throughout their entire lifespan to meet the normal physiological requirements of blood cell turnover, as well as respond to needs for increased demand such as injury or infection. In an average sized human adult it is estimated that approximately 1 trillion blood cells are produced every day, including 200 billion erythrocytes (red blood cells (RBCs)) and 70 billion neutrophils (Ogawa, 1993). This life long process of continuous formation and turnover of blood cells is termed hematopoiesis. How hematopoiesis is regulated remains a subject of debate. However, it is generally accepted that both stochastic and instructive mechanisms play active roles in maintaining hematopoiesis. This chapter will provide a brief overview of the key findings that have contributed to the understanding of the capacity for lifelong blood production, with a focus on regulation of this physiological process by eicosanoids.

Hematopoiesis – Stem and progenitor cells and colony assays:

At the center of lifelong blood cell production is the hematopoietic stem cell (HSC), which has the capacity to give rise to all mature circulating blood cells, i.e., erythrocytes, platelets, lymphocytes, monocytes/macrophages, and neutrophilic, eosinophilic, and basophilic granulocytes. HSCs are defined by two fundamental characteristics: the ability to self-renew, a divisional event which

results in two HSCs, and the ability to differentiate into all mature blood lineages, known as multipotency. Key experiments dating back to the early 1950s (Jacobson et al., 1950; Jacobson et al., 1951; Lorenz et al., 1951) demonstrated that hematopoiesis in irradiated animals could be restored with spleen and/or bone marrow (BM) derived cells. Shortly thereafter, it was demonstrated that allogeneic skin grafts were tolerated in mice who had received lethal irradiation followed by a hematopoietic transplant (Main and Prehn, 1955), leading to the concept of chimerism, in which the donor cells were reconstituting the irradiated host, which was confirmed in later studies (Ford et al., 1956; Nowell et al., 1956; Trentin, 1956). In the 1960's, Till and McCulloch and colleagues published breakthrough studies showing that single clonogenic cells existed within the bone marrow that could self-renew and restore hematopoiesis (Becker et al., 1963; Leung et al., 2007; Siminovitch et al., 1963; Till and McCulloch, 1961; Wu et al., 1967; Wu et al., 1968), thus postulating the *in vivo* existence of a hematopoietic stem cell. These assays utilized lethally irradiated mice that were injected with hematopoietic cells and analyzed macroscopic nodules that formed on the spleens in proportion to the number of bone marrow cells injected (Till and McCulloch, 1961). They hypothesized that the spleen colonies (colony-forming units-spleen (CFU-S)) were derived from a single cell, which they later demonstrated by analysis of chromosomal markers (Becker et al., 1963). These studies also laid the groundwork for clinical hematopoietic transplantation.

Once the existence of cells capable of reconstituting lethally irradiated hosts was identified, it became important to develop methods of isolating and characterizing these cells for further analysis. The pivotal development of *in vitro* assay systems for culturing hematopoietic cells by Bradley and Metcalf (Bradley and Metcalf, 1966), Ichikawa et al. (Ichikawa et al., 1966) and Pluznik and Sachs (Pluznik and Sachs, 1966), with refinements for long-term cultures by Dexter et al. (Dexter et al., 1977) and Whitlock et al. (Whitlock et al., 1984), allowed many of the developmental pathways involved in hematopoietic homeostasis to be identified and the regulatory molecules directing this process to be identified and ultimately cloned. The colony-forming cell assays identify populations of cells with distinct lineage-restricted differentiation patterns. These lineage-restricted cells, or hematopoietic progenitor cells (HPCs), were characterized by the type of colonies they could form in semi-solid agar, methylcellulose or plasma clot. The earliest colonies described were from normal humans and leukemic patients, primarily myeloid derived, and were generally termed colony forming cells (CFCs) (Greenberg et al., 1971; Harris and Freireich, 1970; Haskill et al., 1970; Iscove et al., 1971; Metcalf et al., 1969; Metcalf and Moore, 1970; Senn et al., 1967) and were stimulated to proliferate by various forms of conditioned media. Later, more specific assays identified colony-forming units-granulocytes (CFU-G), colony-forming units-monocytes/macrophages (CFU-M), and colony forming units-granulocytes/macrophages (CFU-GM). Erythroid colony cultures were first identified in 1971 (Stephenson et al., 1971) with subsequent refinements which

identified the more immature burst-forming unit-erythroid (BFU-E) and the immediate precursor of erythroblasts, the colony-forming unit-erythroid (CFU-E) (Gregory, 1976; Heath et al., 1976; Iscove and Sieber, 1975; McLeod et al., 1974). These colonies were determined to be clonally derived (Prchal et al., 1976) and functionally distinct, establishing the beginnings of a hierarchical model in which BFU-E were an earlier, more immature progenitor, while CFU-E were more committed erythroid progenitors, or erythroblasts (Chui et al., 1978; Heath et al., 1976). Related to the erythroid colony assays, identification of a megakaryocyte-restricted colony forming unit was described (Metcalf et al., 1975a; Nakeff and Daniels-McQueen, 1976) (now termed CFU-Mk or CFU-Meg), and the identification of progenitors with multipotential were described (Fauser and Messner, 1978; Fauser and Messner, 1979; Hara and Ogawa, 1978; Johnson and Metcalf, 1977; McLeod et al., 1976), the most common one assayed today referred to as a colony-forming unit granulocyte/erythrocyte/macrophage/megakaryocyte (CFU-GEMM). Culture systems to define the B-lymphoid and T-lymphoid lineages are significantly more difficult to perform, though *in vitro* assays have been developed and used by some investigators to quantitate lymphopoietic potential (Metcalf et al., 1975b; Metcalf et al., 1975c; Schmitt and Zuniga-Pflucker, 2002; Whitlock and Witte, 1982). However, while these various *in vitro* culture assays have been the gold standard in defining HPC function, they do not measure HSCs.

Hematopoiesis – Competitive transplantation assays:

Initially, the CFU-S assay (Till and McCulloch, 1961) was believed to measure HSCs, and is still used by many investigators today as a surrogate HSC assay. Other surrogate assays commonly used to imply HSC function include the cobblestone area-forming cells (CAFC) assay (Ploemacher et al., 1989; Ploemacher et al., 1991; Ploemacher et al., 1993) and the long-term culture-initiating cell (LTC-IC) assay (Lemieux et al., 1995; Sutherland et al., 1989; Sutherland et al., 1990). While these assays may certainly be indicative of a more immature population of cells than the aforementioned CFC and CFU assays, they are not definitive assays for HSC function (Harrison, 1972; Kretchmar and Conover, 1970). The only true measure of HSC function is the ability to fully repopulate a lethally irradiated host. By this definition, the “presence” of HSCs could be determined just by monitoring survival of lethally irradiated transplant recipients. If the mice lived (longer than 16 weeks), with reconstitution of all blood lineages, then by definition, the graft must have contained HSCs. However, this strict “survival” method does not allow for the ability to quantify HSC number or function, and limits the ability to compare HSC grafts. To address this problem, various types of long-term repopulation assays, which assess long-term repopulating cells (LTRC), an HSC synonym, with comparison against a “competitor” graft were developed. The standard competitive HSC repopulation assay was first described by Harrison (Harrison, 1980) followed by description of a calculation for competing repopulating units

(RU) (Harrison et al., 1993) that are one measure used to enumerate HSC. In this assay, a donor HSC graft is admixed with a competing bone marrow graft from a congenic, wild-type mouse, and the mixture is transplanted into a lethally irradiated recipient. Markers distinct for the donor graft and the competitor graft are then used to distinguish blood production from each source of cells, allowing for a comparison of the repopulating ability of each. The standard method of employing this technique today uses the C57Bl/6 (CD45.2) mouse and the B6.SJL-PtrcAPep3B/BoyJ (BOYJ) (CD45.1) mouse. These strains of mice only differ at the CD45 antigen, and can be distinguished with specific monoclonal antibodies, allowing for assessment of chimerism in recipient animals (Shen et al., 1986). A variation of this assay is the limiting-dilution competitive repopulation assay, in which a series of dilutions of the donor, or “test” graft, is compared to a standard number of competing cells (normally 2×10^5 whole bone marrow cells). A minimum threshold of reconstitution is set (~2-5%) and the number of mice that do not reconstitute with the test graft is determined and the frequency of competitive repopulating units (CRU), or HSC, contained within the test graft determined by Poisson statistics (Szilvassy et al., 1989; Szilvassy et al., 1990; Taswell, 1981). It has recently been suggested by Drs. Purton and Scadden that a nomenclature distinction between RU and CRU should be made to describe the above transplantation assays (Purton and Scadden, 2007); however, to date, CRU is still commonly used in both instances.

Typically, competitive transplantation assays are analyzed 12-16 weeks post-transplant, and if multi-lineage reconstitution is seen at this time point, it is assumed that HSC were transplanted. However, it is becoming increasingly clear that HSCs are a heterogeneous population with varying capacities for self-renewal, and as a consequence, varying capacities for extended repopulation. Early studies analyzing CFU-S after serial transplantation hinted at a reduction in self-renewal ability following multiple transplants (Vos and Dolmans, 1972), and serial transplantation was used by others to assess the potential of “younger” HSC (Lemischka et al., 1986; Ogden and Mickliem, 1976; Rosendaal et al., 1979). It was found that in normal mice, the ability of HSC to self-renew is lost after four or five serial transplantations (Ogden and Mickliem, 1976). Recently, experimental evidence indicates the presence of three classes of HSC that differ in the ability to self-renew and the capacity for multi-potent differentiation into all blood lineages: short-term HSC (ST-HSC) capable of full reconstitution for up to 16 weeks, intermediate-term HSC (IT-HSC) capable of full reconstitution for up to 32 weeks, and long-term HSC (LT-HSC) capable of reconstitution for longer than 32 weeks and/or through serial transplantation (Benveniste et al., 2010). In light of these various potentials for self-renewal, the most stringent test of HSC potential, specifically the LT-HSC, is serial transplantation from primary recipients into secondary recipients, or beyond.

While the congenic mouse competitive repopulation assays are suitable for analysis of murine HSC, they do not allow for analysis of human HSC.

Instead, human hematopoietic cells have been analyzed in immunocompromised mice (McCune et al., 1988) and HSC analyzed in an analogous model to the competitive repopulation assay by transplantation into sublethally irradiated non-obese diabetic severe combined immunodeficient (NOD/SCID) mice (Conneally et al., 1997; Lapidot et al., 1992). Similar to the mouse congenic assay, assessment of chimerism and multi-lineage reconstitution is performed to evaluate human HSC function. Recently, a variation of this strain introducing a knockout of the interleukin-2 (IL-2) receptor gamma chain (referred to in this dissertation as an NSG mouse) was developed that shows significantly enhanced engraftment of human HSC, allowing for a better model of human hematopoiesis (Ito et al., 2002). Engraftment of human HSC *in utero* in fetal sheep has also been used successfully as a highly predictive experimental model of human hematopoiesis (Flake et al., 1986; Srour et al., 1993; Zanjani et al., 1992); however, due to the considerable expense and technical considerations of this model compared to SCID mice, it is less widely utilized.

Hematopoiesis – Immunophenotypic characterization of HSC and HPC:

So far, this discussion on stem and progenitor identity has focused on experimental assays to determine HSC and HPC that are all direct or indirect measures of the functional ability of these cells; whether it is the ability to form macroscopic colonies on the spleen, lineage specific colonies in media, or repopulation of lethally irradiated recipients. In addition to these functional assays, immunophenotypic analysis is commonly used to determine the number

or frequency of HSC and HPC, and used as a means to “sort” specific populations for further experimentation. Immunophenotypic analysis utilizes antigen specific antibodies coupled with fluorescent labels and fluorescence-activated cell sorting (FACS) that is able to rapidly enumerate and/or collect specific cell populations. Early work on immunophenotyping hematopoietic populations began in the Weissman lab where they demonstrated that mature B cells and their immediate precursors could be defined by a specific antibody (Coffman and Weissman, 1981), which has lead to a set of lineage markers (Lin) to define mature blood cells including erythrocytes, granulocytes, macrophages, T-cells, B-cells, natural killer (NK) cells and megakaryocytes, and lineage negative cells that are enriched for earlier stem and progenitor populations (Muller-Sieburg et al., 1986). Later, it was demonstrated that repopulating cells could be further defined by the absence of lineage markers (Lin^{neg}) with expression of stem cell antigen-1 (Sca-1) and low expression of Thy1.1 (Spangrude et al., 1988). Later, an additional marker for the stem cell factor (SCF) receptor (c-kit) (Ikuta and Weissman, 1992; Ogawa et al., 1991; Okada et al., 1992) was added to further define the murine HSC population. Throughout this dissertation, these cells will be referred to as Sca-1⁺ c-kit⁺ Lin^{neg} (SKL) cells. While enriched for HSC function, SKL cells are still heterogeneous. Additional markers have recently been identified to further enrich for HSCs, including CD34 (Osawa et al., 1996), and *fms*-related tyrosine kinase-3 (Flt3) (Adolfsson et al., 2001; Yang et al., 2005), that allow for the characterization of LT-HSC (CD34⁻

Flt3⁻ SKL), ST-HSC (CD34⁺ Flt3⁻ SKL) and multi-potent progenitors (MPPs) (CD34⁺ Flt3⁺ SKL) (Yang et al., 2005). Several additional markers have now been identified that further refine HSC identity, including Endoglin (CD105) (Chen et al., 2002; Chen et al., 2003), Tie2 (CD202) (Arai et al., 2004), Endothelial protein C receptor (CD201) (Balazs et al., 2006), CD49b (Benveniste et al., 2010) and notably the signaling lymphocyte activation molecule (SLAM) family of receptors CD150, CD48 and CD244 (Kiel et al., 2005; Yilmaz et al., 2006). Throughout this dissertation, CD150⁺ CD48⁻ SKL, which are highly enriched for LT-HSC (Chen et al., 2008), will be referred to as SLAM SKL cells.

While there exists a comprehensive list of markers to define murine HSC, there are relatively few immunophenotypic markers for human HSC. The first marker identified for human HSC and HPC was the CD34 antigen (Andrews et al., 1986; Batinic et al., 1989; Civin et al., 1984; Watt et al., 1987), and cells enriched for CD34 were found to be able to repopulate lethally irradiated baboons (Berenson et al., 1988). Although the CD34 cell population is very heterogeneous, it is used clinically as an assessment of hematopoietic graft quality/quantity (Gratama et al., 1998; Krause et al., 1996). However, it should be noted that not all HSC are CD34⁺ (Bhatia et al., 1998; Donnelly and Krause, 2001; Zanjani et al., 1998) and the ability of CD34⁻ cells to give rise to CD34⁺ cells has been demonstrated (Nakamura et al., 1999; Zanjani et al., 1998). The ability of transplanted CD34⁺ cells to give rise to CD34⁻ cells capable of regenerating CD34⁺ cells (Dao et al., 2003) further indicates that CD34 is not an

exclusive marker of human HSC. Analogous to the murine SKL population, additional markers have been identified in coordination with CD34 to further define human HSC. Human bone marrow cells that were CD34⁺, and negative for histocompatibility lymphocyte antigen-DR (HLA-DR)^{neg} were found to contain primitive cells capable of self-renewal and multipotential differentiation in *in vitro* systems (Brandt et al., 1988; Srour et al., 1991), although this marker may differ depending on which hematopoietic tissue cells are extracted from (Huang and Terstappen, 1994; Traycoff et al., 1994). The most widely used purification marker in addition to CD34 for human HSC is the CD38 antigen, where CD34⁺ CD38⁻ cells were shown to be more primitive than their CD38⁺ counterparts (Terstappen et al., 1991), and were later shown to have enhanced SCID repopulating ability (Bhatia et al., 1997) and sustained re-transplantable, human hematopoiesis in human/sheep chimeras (Civin et al., 1996). Additionally, CD90 (Thy-1) has been shown to enrich for HSC activity within the CD34⁺ population in animal models and in clinical trials (Baum et al., 1992; Murray et al., 1995; Negrin et al., 2000; Sutherland et al., 1996; Vose et al., 2001). AC133 has been suggested as a potential HSC marker (Yin et al., 1997), and recently it was shown that AC133 may be a more reliable HSC marker for *ex vivo* expanded human umbilical cord blood (UCB) (Ito et al., 2010).

Hematopoiesis – The “hematopoietic tree”:

While it is clear that the hematopoietic system contains single hematopoietic stem cells that can give rise to all lineages contained within blood,

the pathway of lineage restricted differentiation patterns that ultimately give rise to these mature cells is less clear. Early on, differentiation patterns were based on microscopic observation of the morphological features of blood cells, where precursors for red cells, platelets, monocytes and granulocytes could be distinguished in terms of shape or staining properties (Kawamoto and Katsura, 2009). With the advent of clonogenic colony assays and immunophenotypic marking, considerable research has been spent isolating cells and determining their capacity to self-renew and/or differentiate into specific lineages. This research has led to the descriptions of lineage restricted progenitors, which have been placed along a differentiation hierarchy, or “hematopoietic tree” (Figure 1). Some of these progenitors include the common lymphoid progenitor (CLP), common myeloid progenitor (CMP), granulocyte-monocyte progenitor (GMP), and the megakaryocyte-erythrocyte progenitor (MEP) (Akashi et al., 2000; Kondo et al., 1997), which were shown to lack self-renewal capabilities (Na et al., 2002) and have been placed in “branches” of the hematopoietic tree (Shizuru et al., 2005). In addition, common myelolymphoid (CMLP) and common myeloerythroid (CMEP) progenitors have been described, which blur the once commonly held line between strict myeloid and lymphoid branches in the hematopoietic tree. MPPs upstream of these progenitors have also been identified with limited self-renewal capacity (Adolfsson et al., 2005; Christensen and Weissman, 2001; Morrison and Weissman, 1994), which in many cases may be functionally indistinguishable from ST-HSCs. Phenotypic markers which can

enrich for each of these progenitor populations have been identified, and are thoroughly reviewed (Weissman and Shizuru, 2008). These and other findings have lead to numerous models of hematopoietic trees including models that suggest random differentiation to various lineages (Ogawa et al., 1983); a sequential determination model in which MPPs progressively differentiate into progenitors with erythroid, myeloid, B and T cell lineage potentials (Brown et al., 1985; Brown et al., 2007); a model based on transcription factor expression that indicates an early split to progenitors with erythroid potential, with development of myeloid and lymphoid progenitors further down the tree (Singh, 1996); a model which incorporates the findings of a CMLP (Adolfsson et al., 2005); and numerous other variations. Studies evaluating the potential of ST-HSCs or LT-HSCs have also found variations in lineage commitment, where a fraction of SKL cells was shown to preferentially differentiate into lymphoid lineages (Igarashi et al., 2002), and elegant single-cell transplant studies have identified balanced (Bala), Lymphoid-biased (Ly-bi) and Myeloid-biased (My-bi) HSCs. While each of the three subsets of HSCs give rise to all blood lineages, the proportions of lineages produced is skewed in pre-determined patterns (Muller-Sieburg et al., 2002; Muller-Sieburg et al., 2004; Muller-Sieburg and Sieburg, 2006a; Muller-Sieburg and Sieburg, 2006b). Additionally, recent work has shown heterogeneity within the LT-HSC pool, in which some LT-HSC are extremely dormant, but during times of stress can switch out of dormancy to give rise to HSC with increased proliferative potential (Wilson et al., 2008).

Figure 1

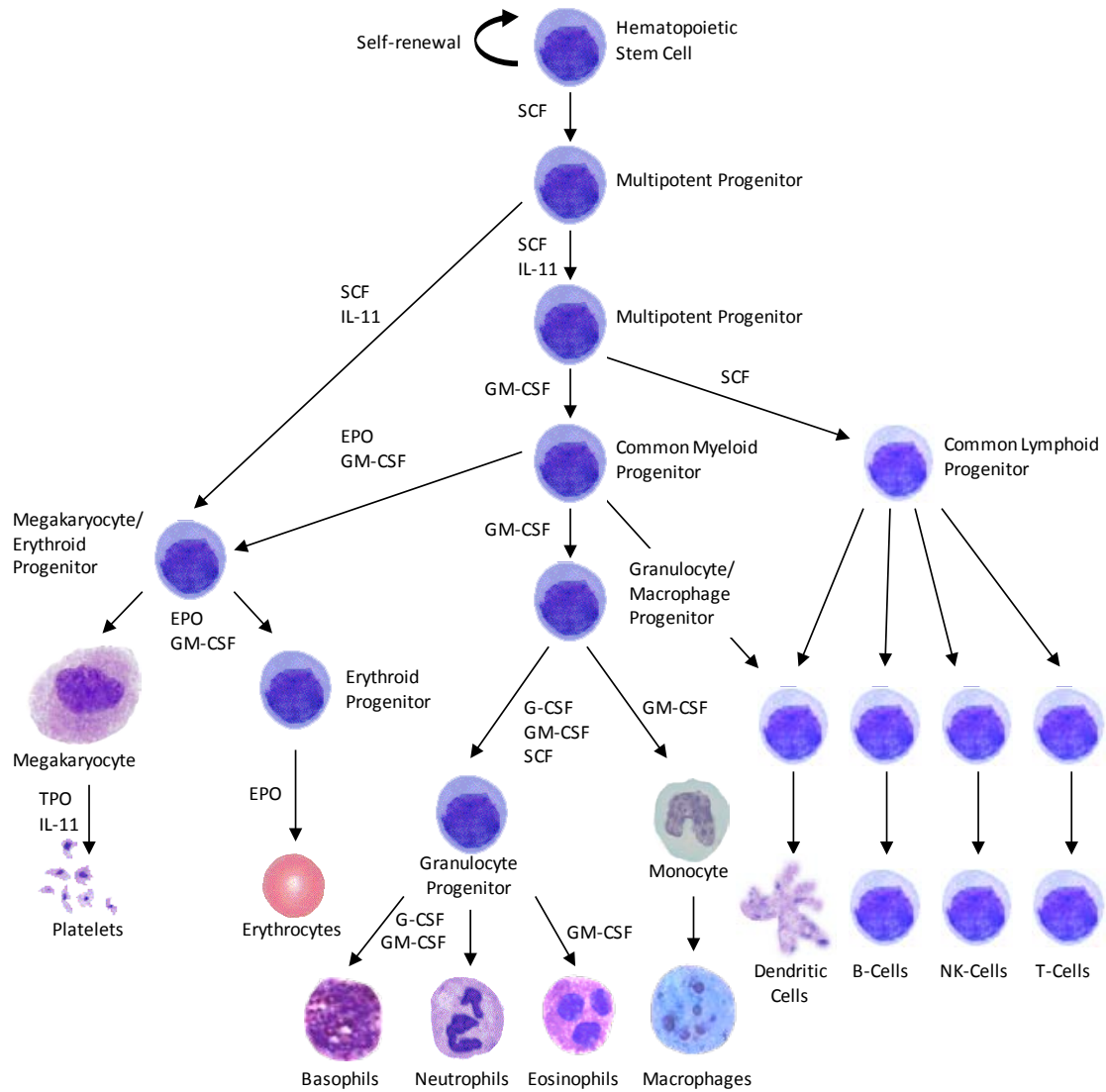


Figure 1. The hematopoietic tree.

Blood cell development is a hierarchical process with self-renewal and maturational divisions occurring as a continuum under the direction of single or multiple growth factors. Shown is a simplistic representation incorporating current understandings of the hematopoietic process, although clear discrepancies in any model with the body of literature exist. The points of action of cytokines and growth factors that currently have Food and Drug Administration (FDA) approved pharmaceuticals which mimic or antagonize the endogenous pathways are shown, suggesting possible clinical interventions.

Hematopoiesis – The hematopoietic “niche”:

As discussed earlier, control of HSC and HPC proliferation and differentiation is highly complex, and homeostatic balance is likely maintained by both intrinsic and genetic cues within individual cells and extrinsic cues from the supportive microenvironment in which HSCs reside. It was first postulated in 1978 that HSCs reside in very defined and limited microenvironments, or “niches” (Schofield, 1978), and signals within these niches direct HSC maintenance. In mammals, the primary HSC niche is contained within the bone marrow, which is comprised of stromal cells and an extracellular matrix of collagens, fibronectin, and proteoglycans (Yoder and Williams, 1995). Recent studies have shown that osteoblasts within the endosteal bone marrow niche are a significant regulatory component of hematopoiesis (Arai et al., 2004; Calvi et al., 2003; Visnjic et al., 2004; Zhang et al., 2003). Activation of the parathyroid hormone (PTH) receptor in osteoblasts expands HSC by ~4-fold (Taichman et al., 2000; Taichman and Emerson, 1994), and co-transplantation of osteoblasts with HSC increases engraftment (El Badri et al., 1998). The effect of osteoblasts on HSC expansion may be mediated by the Notch ligand Jagged1. Notch signaling regulates cell fate decisions including HSC self-renewal (Karanu et al., 2000; Stier et al., 2002; Varnum-Finney et al., 2003), thus by changing self-renewal versus differentiation decisions, Notch can increase HSC number without differentiation or increase in HPC or mature cells (Karanu et al., 2000; Stier et al., 2002). Studies monitoring trafficking of labeled HSCs transplanted into recipients

have also indicated that HSCs reside within the endosteal niche (Lo et al., 2009; Nilsson et al., 2001; Xie et al., 2009), further validating earlier findings (Gong, 1978; Lord et al., 1975).

Within the niche, HSCs are thought to be “tethered” to osteoblasts, other stromal cells, and the extracellular matrix through a variety of adhesion molecule interactions, many of which are likely redundant systems. Early studies exploring the role of osteoblasts in maintaining HSCs suggested that N-cadherin interactions mediated the positive effects on HSCs (Zhang et al., 2003), however more recent studies have contradicted these findings (Kiel et al., 2007; Kiel et al., 2009). Numerous other adhesion molecules have been implicated as contributing to HSC and HPC tethering, including, but not limited to, the integrins $\alpha 4\beta 1$ – very late antigen-4 (VLA-4) (Levesque et al., 1995; Levesque et al., 2001; Papayannopoulou et al., 2001; Peled et al., 2000; Vermeulen et al., 1998; Zanjani et al., 1999), $\alpha 5\beta 1$ – very late antigen-5 (VLA-5) (Levesque et al., 1995; Peled et al., 2000; van der Loo et al., 1998; Vermeulen et al., 1998), $\alpha 4\beta 7$ – lymphocyte Peyer’s patch adhesion molecule-1 (LPAM-1) (Katayama et al., 2004), the alpha 6 integrins (Laminins) (Qian et al., 2006; Qian et al., 2007), CD44 (Schmits et al., 1997; Vermeulen et al., 1998), E-selectins (Frenette et al., 1998; Katayama et al., 2003; Sackstein, 2004), the angiopoietin receptor tyrosine kinase with immunoglobulin-like and EGF-like domains-2 (Tie-2) (Arai et al., 2004), osteopontin (OPN) (Grassinger et al., 2009; Nilsson et al., 2005), endolyn

(CD164) (Forde et al., 2007), and the calcium-sensing receptor (CaR) (Adams et al., 2006).

The most explored niche interaction, and perhaps the most important in regulating HSC and HPC trafficking to and from the marrow niche, is the interaction between the CXC chemokine receptor 4 (CXCR4) and its ligand stromal cell-derived factor-1 α (SDF-1 α). SDF-1 α is produced by osteoblasts (Ponomaryov et al., 2000), a specialized set of reticular cells found in endosteal and vascular niches (Sugiyama et al., 2006), and SDF-1 α has also been found on endothelial cells and within bone itself (Katayama et al., 2006; Sipkins et al., 2005). HSC and HPC express CXCR4 and are chemo-attracted to and retained within the bone marrow by SDF-1 α (Aiuti et al., 1997; Jo et al., 2000; Kim and Broxmeyer, 1998). In addition to its chemoattraction and retention effects, CXCR4 signaling has been shown to have positive effects on HSC and HPC survival and proliferation (Broxmeyer et al., 2003; Broxmeyer et al., 2005a; Broxmeyer et al., 2007a; Lataillade et al., 2000; Lee et al., 2002). Mice with a functional knock-out of either SDF-1 α or CXCR4 die before birth due to bone marrow failure (Nagasawa et al., 1996; Zou et al., 1998).

While it is clear that HSCs reside and thrive within the endosteal bone marrow niche, evidence suggests that this niche is not the exclusive niche for hematopoiesis. Studies assessing the localization of SLAM cells within the bone marrow have shown a greater proportion of SLAM cells adjacent to the endothelium of bone marrow sinusoidal blood vessels, suggesting a “vascular

niche” for HSC (Kiel et al., 2005; Kiel et al., 2007). This is supported by the fact that endothelial cells, similar to osteoblasts, can support hematopoiesis both *in vitro* and *in vivo* (Li et al., 2004; Ohneda et al., 1998; Yao et al., 2005). While it is not completely clear what factors influence the particular niche location of HSCs, the fact that reticular cells with high production of SDF-1 α are present in both the endosteal and vascular niches, may provide a common mechanism of HSC support in both niches (Kiel and Morrison, 2008; Sugiyama et al., 2006).

Hematopoietic Trafficking – Homing and mobilization:

Hematopoietic stem cell transplantation is routinely used to treat leukemias, cancer, hematologic diseases and metabolic disorders (Kondo et al., 2003). Sources of HSC for transplant include bone marrow (Goldman and Horowitz, 2002), mobilized peripheral blood (Fruehauf and Seggewiss, 2003; Papayannopoulou, 2004; Ringden et al., 2000) and umbilical cord blood (Benito et al., 2004; Broxmeyer et al., 1991; Broxmeyer, 2005; Broxmeyer et al., 2006; Broxmeyer and Cooper, 1997; Gluckman et al., 1989). Hematopoietic reconstitution is a multi-step process, but with some sources of HSCs, efficacy is limited by inadequate HSC number, inability to migrate/home to marrow niches, and poor engrafting efficiency and self-renewal (Broxmeyer, 2006; Hall et al., 2006; Porecha et al., 2006). As discussed earlier, an appropriate bone marrow niche is required for HSC to self-renew and differentiate and only HSC homing to this bone marrow niche are able to repopulate a lethally irradiated recipient long-term (Lanzkron et al., 1999; Nibley and Spangrude, 1998). Homing, in the

context of this dissertation, is a description of the ability of HSC and HPC to traffic from the peripheral blood, where they were injected intravenously, to the bone marrow niche, where they can then successfully repopulate the host. Homing is a rapid process, which is measured in hours (or at most 1-2 days) (Lapidot et al., 2005), and should be separated from the concept of “engraftment”, which is more a description of the culmination of events pre- and post-homing of HSC. While it is necessary for HSC to home to the bone marrow in order to repopulate a lethally irradiated recipient, there is considerable debate as to whether HSC specifically home to the marrow (Benveniste et al., 2003; Kollet et al., 2001; Matsuzaki et al., 2004), or whether HSC simply randomly seed organs in the body (Cui et al., 1999; van Hennik et al., 1999). Our data, presented in Chapter 2 and recently published (Hoggatt et al., 2009), clearly indicate the ability to specifically enhance the homing of SKL cells, without alterations in total bone marrow cell homing, which suggests HSCs have some ability to selectively home to the bone marrow compared to their non-HSC counterparts. Homing of HSC to bone marrow is regulated by many of the same processes previously described that regulate HSC tethering in the bone marrow niche. Adhesion molecules aid in trafficking, leukocyte rolling, transendothelial migration, and ultimate tethering in the marrow, and many of them are essential for proper homing of HSC (Katayama et al., 2004; Kollet et al., 2001; Matsuzaki et al., 2004; Peled et al., 2000; Qian et al., 2006; Qian et al., 2007; van der Loo et al., 1998). Similarly, the CXCR4 and SDF-1 α axis is a critical component of

HSC homing to the bone marrow (Peled et al., 1999), and increases in CXCR4 receptor expression on HSC either with growth factors (Kollet et al., 2001), or by gene overexpression (Brenner et al., 2004; Kahn et al., 2004) significantly increases homing and engraftment. Further discussion on hematopoietic homing will follow in Chapter 2.

Under steady state conditions, HSC and HPC normally reside within the bone marrow niches, while the mature cells produced by these populations ultimately exit the marrow and enter the peripheral blood. However, considerable evidence over the last several decades clearly demonstrates that HSC and HPC also exit the bone marrow niche and traffic to the peripheral blood (Abkowitz et al., 2003; Chervenick and Boggs, 1971; Goodman and Hodgson, 1962; Massberg et al., 2007; McKinney-Freeman and Goodell, 2004; Wright et al., 2001), and this steady state trafficking leaves open niche spaces that can be repopulated by transplanted HSC (Bhattacharya et al., 2009). Based on observations that increased HPC were found in patients after chemotherapy (Kurnick and Robison, 1971; McCredie et al., 1971), we now know that this natural egress of HSC and HPC into the periphery can be enhanced, allowing for “mobilization” of these cells from the bone marrow to the peripheral blood (Abkowitz et al., 2003; Wright et al., 2001). Mobilized adult HSC and HPC are widely used for autologous and allogeneic transplantation and have improved patient outcomes when compared to bone marrow. Mobilization can be achieved through administration of chemotherapy (Kessinger and Armitage, 1991; Kurnick

and Robison, 1971; McCredie et al., 1971), or hematopoietic growth factors, chemokines, or small molecule inhibitors or antibodies against the aforementioned niche chemokine receptors and integrins (Fruehauf and Seggewiss, 2003; Papayannopoulou, 2004; To et al., 1997).

The hematopoietic growth factor, granulocyte-colony stimulating factor (G-CSF) is widely used clinically to mobilize HSC and HPC for transplantation. G-CSF is a glycoprotein that binds to a type 1 cytokine receptor (G-CSFR) to stimulate proliferation (Duhrsen et al., 1988; Welte et al., 1987) and differentiation (Souza et al., 1986). G-CSF can also support proliferation of other types of progenitor cells in combination with other growth factors (Ikebuchi et al., 1988a; Ikebuchi et al., 1988b; Metcalf and Nicola, 1983). G-CSF-mobilized HSC and HPC are associated with more rapid engraftment, shorter hospital stay (Jansen et al., 1999; Kennedy et al., 1993; McQuaker et al., 1997; Nemunaitis et al., 1995), and in some circumstances, superior overall survival compared to bone marrow (Stem Cell Trialists' Group, 2005). Normal administration of G-CSF for mobilization of HSC and HPC is 5-7 days to patients and normal donors; however, this administration is often associated with morbidity in the form of bone pain, nausea, headache and fatigue (Anderlini et al., 1998; Anderlini et al., 2001; Fortanier et al., 2002; Rowley et al., 2001), which can be lifestyle disruptive in normal volunteers. G-CSF has also been associated with serious toxicity, including enlargement of the spleen (Platzbecker et al., 2001; Stroncek et al., 2003) and splenic rupture (Balaguer et al., 2004; Becker et al., 1997; Falzetti et

al., 1999; Kroger et al., 2002) in normal donors, and the pro-coagulant effects of G-CSF can increase the risk of myocardial infarction and cerebral ischemia in high-risk individuals (Hill et al., 2005; Lindemann and Rumberger, 1993). Despite its dramatic effect on the field of hematopoietic transplantation, poor mobilization in response to G-CSF occurs in 25% of patients, particularly those with lymphoma and multiple myeloma (Stiff et al., 2000) and 15% of normal donors (Anderlini et al., 1997), requiring extended aphereses (Schmitz et al., 1995). In addition, the incidence of chronic graft-versus-host disease (GVHD) is higher (Couban et al., 2002; Cutler et al., 2001; Mohty et al., 2002) for G-CSF-mobilized HSC and HPC when compared to grafts obtained from bone marrow.

Although G-CSF is the predominate mobilizer used clinically, other mobilizers with apparent different mechanisms of action have been described, including chemokine agonists of the CXCR2 receptor (Fukuda et al., 2007; King et al., 2001; Pelus et al., 2004; Pelus et al., 2006a; Pelus and Singh, 2008), small molecule or antibody inhibitors of the VLA-4 and vascular cell adhesion molecule-1 (VCAM-1) interaction (Ramirez et al., 2009; Zohren et al., 2008), sulfated glycans (fucoidan) which inhibit adhesion through selectins and increase plasma SDF-1 α (Frenette and Weiss, 2000; Sweeney et al., 2002), and antagonism of the CXCR4 receptor (Broxmeyer et al., 2005b; Broxmeyer et al., 2007b; Devine et al., 2008; Liles et al., 2003; Liles et al., 2005; Pelus et al., 2005), to name a few. Many of these compounds work in synergy with G-CSF to increase mobilization of HSC and HPC (Kikuta et al., 2000; Liles et al., 2005;

Pelus et al., 2006a; Pelus et al., 2006b; Velders et al., 2002). In fact, the CXCR4 antagonist AMD3100 (Mozobil™) received FDA approval in December 2008 and is indicated for mobilization of HSC and HPC in combination with G-CSF for patients with non-Hodgkin's lymphoma and multiple myeloma. Despite the success of G-CSF and AMD3100, there is considerable room for improvement in mobilization of HSC and HPC, not only to increase hematopoietic yield, but also to reduce/avoid the known toxicities of G-CSF. Novel mobilization strategies are of clinical interest and highly significant, and are a focus of Chapter 3.

Eicosanoid Biosynthesis:

The focus of this dissertation is on the ability of eicosanoids to regulate hematopoiesis, and to alter eicosanoid signaling for therapeutic utility. Eicosanoids include the prostaglandins (PGs) along with prostacyclins, thromboxanes, leukotrienes and endocannabinoids, which are formed by oxidation of 20-carbon essential fatty acids released from phospholipids. Eicosanoids affect every organ, tissue, and cell in the body (Funk, 2001). These signaling molecules are all derived from the oxidation of 20-carbon essential fatty acids; hence their name, from the Greek *Eikosi* meaning 20. The notion that fatty acids could act as physiological regulators originates with the work of Burr, G. and Burr, M. in which they described a wide array of physiological effects in rats whose diet was completely devoid of fats (Burr and Burr, 1930). Their observations lead to the concept of “essential fatty acids” and paved the way for lipid research. At around the same time, work by Kurzrock and Lieb reported that

human semen caused contraction of myometrium *in vitro* (Kurzrock and Lieb, 1930). These findings were later supported by independent observations from Goldblatt (Goldblatt, 1935) and Von Euler (Von Euler, 1936) and Von Euler called the active substance “prostaglandin” with the belief that it was formed by the prostate gland. Over the course of the next 30 years, pioneering work by Bergstrom, Samuelsson, Moncada and Vane led to further understanding of prostaglandin biosynthesis and tissue origins (Flower, 2006; Miller, 2006), and the structure of prostaglandin E and its derivation from the previously described essential fatty acids was determined (Bergstrom, 1967).

Prostaglandin E₂ (PGE₂) is the most abundant eicosanoid (Murakami and Kudo, 2006; Serhan and Levy, 2003) and a known mediator of cancer (Hull et al., 2004; Murakami and Kudo, 2006), fever (Ivanov and Romanovsky, 2004; Lazarus, 2006), inflammation (Murakami and Kudo, 2006; Samuelsson et al., 2007), atherosclerosis, blood pressure and strokes (Samuelsson et al., 2007), ovulation (Davis et al., 1999) and numerous other physiological systems (Miller, 2006). Prostaglandins are synthesized in 3 steps (Figure 2): cleavage of arachidonic acid from phospholipids by phospholipase A₂; oxidation by cyclooxygenase enzymes (COX1 and COX2) forming the unstable intermediate PGG₂, followed by reduction to form PGH₂; and isomerization to mature PGs by specific synthases (Ivanov and Romanovsky, 2004; Miller, 2006; Murakami and Kudo, 2004; Park et al., 2006; Smith et al., 1991). All nucleated cells synthesize PGs (Miller, 2006). There are three main classes of phospholipase A₂ (PLA₂):

secreted PLA₂, intracellular group VI calcium-independent PLA₂, and group IV cytosolic PLA₂ (cPLA₂) (Park et al., 2006). Although each of these PLA₂ enzymes are able to release arachidonic acid from membrane phospholipids, cPLA₂ is the predominate phospholipase responsible for induced eicosanoid production (Funk, 2006; Leslie, 2004; Murakami and Kudo, 2004). Mice deficient in cPLA₂ demonstrate marked reduction in PGE₂ (Uozumi and Shimizu, 2002) and subsequent reduction in airway reactivity (Uozumi et al., 1997), decreased platelet aggregation (Wong et al., 2002), and notably a reduction in collagen-induced arthritis (Hegen et al., 2003). Inactive cPLA₂ is present in the cytosol of resting cells (Ivanov and Romanovsky, 2004) and upon stimulation by inflammatory agents, growth receptors, increased intracellular calcium and other stimulatory signals, cPLA₂ is translocated to the Golgi complex, endoplasmic reticulum, and nuclear envelope where it is activated (Clark et al., 1995; Evans et al., 2001; Ivanov and Romanovsky, 2004). In addition, cPLA₂ has also been shown to be up-regulated by mitogen-activated protein kinases (MAPK) (Clark et al., 1995; Park et al., 2006) and transcriptional upregulation of cPLA₂ in macrophages stimulated by lipopolysaccharides (LPS) was reported (Dieter et al., 2002). Since activation of cPLA₂ determines the amount of free arachidonic acid available, cPLA₂ is the principle regulator of eicosanoid production (Leslie, 2004). Corticosteroids, which are known anti-inflammatory drugs, were once thought to act by physically incorporating into cellular membranes making arachidonic acid less available to cPLA₂, however, corticosteroids may also act to

directly inhibit cPLA₂ via induction of an inhibitory protein, lipocortin (Flower, 1990).

Once arachidonic acid is freed by cPLA₂ it is then available to cyclooxygenase, lipoxygenase (LOX), or other enzymes for metabolism. Cyclooxygenase is a heme-containing enzyme that is responsible for two enzymatic steps in arachidonic acid metabolism: (1) a dioxygenase step which causes the cyclization of arachidonic acid by adding a 15-hydroperoxy group to form the PGG₂ intermediate, and (2) a hydroperoxidase step which reduces the 15-hydroperoxy group of PGG₂ to form the intermediate PGH₂ (Ivanov and Romanovsky, 2004; Miller, 2006; Murakami and Kudo, 2004; Park et al., 2006; Sigal, 1991). COX1 is constitutively expressed and responsible for basal function, while COX2 is induced by inflammatory mediators and cytokines (Funk, 2006; Miller, 2006; Murakami and Kudo, 2004; Park et al., 2006). Differences in the function of the 2 COX enzymes result in differences in kinetics, intracellular localization, (Goetzl et al., 1995; Ivanov and Romanovsky, 2004) and coupling to PG synthases (Ivanov and Romanovsky, 2004). Highly selective COX1 (SC-560), COX2 (celecoxib (Celebrex®), SC-236) and dual (indomethacin (Indocin®)) targeted drugs have been developed (Coruzzi et al., 2007; Mitchell and Warner, 2006; Strand, 2007). The parent compounds are readily available and an extensive literature validates their *in vitro* and *in vivo* utility. Specific PG synthases convert PGH₂ into thromboxanes, prostacyclins (PGI₂), or D, E, or F series PGs (Dupuis et al., 1997; Folco and Murphy, 2006; Funk, 2001; Goetzl et

al., 1995; Park et al., 2006; Urade et al., 1995). The mechanisms that control specific PG formation are not entirely clear (Park et al., 2006); however, regulation is cell type specific (Funk, 2001; Helliwell et al., 2004). In the case of PGE₂, PGH₂ is isomerized by inducible membrane PGE synthase-1 (mPGES-1) regulated downstream of MAPK, although other signaling pathways can be involved (Murakami and Kudo, 2006; Park et al., 2006; Samuelsson et al., 2007). Based on chemical/metabolic instability, PGE₂ is thought to act locally in autocrine or paracrine fashion (Tsuboi et al., 2002). Osteoblasts are a major source of PGE₂ (Chen et al., 1997; Miyaura et al., 2003; Raisz et al., 1979) and due to their physical proximity to HSC in the niche, could likely be a source of PGE₂ involved in paracrine signaling.

The leukotrienes are biosynthesized in two steps by oxygenation of arachidonic acid via the 5'-LOX pathway and conversion to the unstable intermediate leukotriene (LT) A₄ (LTA₄), that is enzymatically hydrolyzed to LTB₄ or conjugated to glutathione forming the cysteinyl leukotriene LTC₄; that is subsequently converted to LTD₄ and LTE₄ (Figure 2). Leukotriene formation occurs predominantly in inflammatory cells, including granulocytes, mast cells and macrophages (Dupuis et al., 1997; Folco and Murphy, 2006; Funk, 2001; Goetzl et al., 1995), however 12'-LOX in platelets can convert LTA₄ produced by granulocytes to LTB₄ and lipoxins (Serhan and Sheppard, 1990). LTB₄ is produced at sites of inflammation and stimulates inflammatory leukocyte function

(Kim and Luster, 2007; Kondo et al., 2003). Like PGs, the leukotrienes have a short half-life and therefore are primarily involved in localized signaling.

The two main endocannabinoids, anandamide and 2-arachidonoyl glycerol (2-AG), are derivatives of arachidonic acid and are synthesized on demand (Ligresti et al., 2005; Malcher-Lopes et al., 2008; Okamoto et al., 2007).

However, since they are structurally similar to arachidonic acid, they are also substrates for COX enzymes (Kozak et al., 2002; Weber et al., 2004; Yu et al., 1997), resulting in alternative PGs, with similar and novel effects (Kozak et al., 2001; Kozak et al., 2002), which can be metabolized into traditional forms via esterases or by dehydration (Kozak et al., 2001). Endocannabinoids can also be metabolized back to arachidonic acid via fatty acid amide hydrolase (FAAH) (Burstein et al., 2000; Ueda and Yamamoto, 2000) (Figure 2). Recycling of endogenous endocannabinoids for new endocannabinoid biosynthesis can also occur (Placzek et al., 2008). Cannabinoids can stimulate the production of PGs (Mitchell et al., 2008), and likewise, PGE₂ can stimulate synthesis of 2-AG intermediates (Konger et al., 2005). In summary, it is clear that eicosanoid biosynthesis is interactive and changes in synthesis or signaling can alter overall eicosanoid balance and produce eicosanoids with similar or opposing functions.

Figure 2

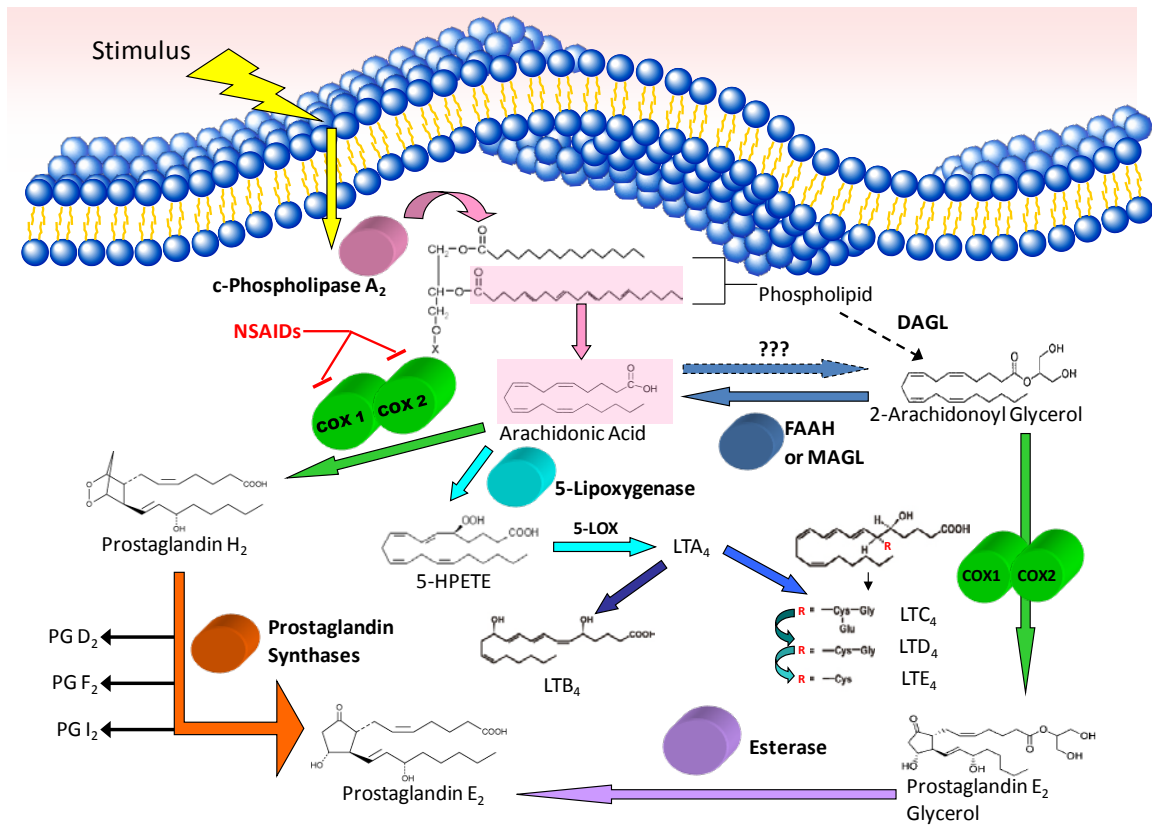


Figure 2. Schematic of eicosanoid biosynthesis.

A stimulus via a G-protein coupled receptor (GPCR) activates c-phospholipase A_2 to release arachidonic acid (AA). Cyclooxygenase (COX) enzymes, which can be inhibited by non-steroidal anti-inflammatory drugs (NSAIDs), convert AA into prostaglandins. Similarly, 5'-lipoxygenase (5'-LOX) converts AA into the intermediate 5-hydroperoxyeicosatetraenoic acid (5-HPETE) and then into the various leukotrienes. Endocannabinoid (2-arachidonoyl glycerol) is synthesized from FAAH, monoacylglycerol lipase (MAGL) or possibly indirectly by diacylglycerol lipase (DAGL), and its metabolism is intricately linked to overall eicosanoid synthesis, and can be converted into alternate prostaglandin forms.

Eicosanoid Receptors and Signaling:

Once synthesized, PGE₂ is able to passively leave the cell or can be actively transported. However, due to its chemical and metabolic instability, PGE₂ is thought to act locally in an autocrine or paracrine fashion (Tsuboi et al., 2002). PGE₂ interacts with 4 conserved G-protein coupled receptors (GPCR); EP1, EP2, EP3, and EP4 (Breyer et al., 2001; Hull et al., 2004; Sugimoto and Narumiya, 2007; Tsuboi et al., 2002) that account for the multiple, sometimes opposing effects attributed to PGE₂ (Breyer et al., 2001) (Figure 3). EP receptor levels vary among tissues, with EP3 and EP4 mRNA being most abundant (Sugimoto and Narumiya, 2007) and EP2 mRNA expressed at lower levels than EP4 in most tissues (Katsuyama et al., 1995). EP1 activates phospholipase C (PLC) via an unidentified G protein (Tsuboi et al., 2002), increasing intracellular Ca²⁺ coupled to inositol phosphates resulting in activation of protein kinase C (PKC) (Breyer et al., 2001; Tsuboi et al., 2002). EP3 activation results in G_{ai}-linked inhibition of adenylate cyclase and decreased cyclic adenosine monophosphate (cAMP) (Lazarus, 2006; Sugimoto and Narumiya, 2007). Multiple EP3 splice variants have been identified that can couple to multiple G proteins (Namba et al., 1993). EP2 and EP4 both activate adenylate cyclase and increase cAMP through G_{as}, activating protein kinase A (PKA) (Breyer et al., 2001; Hull et al., 2004; Sugimoto and Narumiya, 2007; Tsuboi et al., 2002), as well as Rap1, Rac1, and PKCζ (PKC zeta), a unique isoform implicated in HSC function (Goichberg et al., 2006). EP2 and EP4 have partially redundant roles in

some systems, but distinct roles in others (Sugimoto and Narumiya, 2007). EP4, but not EP2, can activate the phosphoinositide 3-kinase (PI3K) Akt pathway (Fujino et al., 2003; Regan, 2003). EP4 has a longer cytosolic domain allowing for more ligand dependent phosphorylation and more rapid desensitization (Nishigaki et al., 1996), enabling a selective negative feedback loop (Sugimoto and Narumiya, 2007). Lastly, EP4 is internalized when activated, while EP2 is not (Desai et al., 2000). As a consequence, EP2 and EP4 can have different roles based upon continuation or attenuation of signals generated by receptor activation (Breyer et al., 2001). PGE₂ often exhibits a “bell-shaped” dose-response curve suggesting a different repertoire of EP receptors is activated dependent upon PGE₂ concentration (Hull et al., 2004). EP2 and EP4 activation is also associated with phosphorylation of glycogen synthase kinase-3 (GSK-3). EP2 inhibits GSK-3 via PKA, whereas EP4 can also inhibit GSK-3 through PI3K (Hull et al., 2004; Regan, 2003). Inhibition of GSK-3 decreases β -catenin phosphorylation, allowing nuclear entry and effects on gene transcription (Cadigan and Nusse, 1997; Khan and Bendall, 2006). Interestingly, β -catenin is downstream of the wingless and INT (Wnt) pathway implicated in HSC survival and self-renewal (Khan and Bendall, 2006). PGE₂ through EP4 can increase β -catenin and studies suggest that COX2 and Wnt pathways exhibit synergistic cross-talk (Wang et al., 2004). Inhibition of GSK-3 and activation of extracellular signal-regulated kinase 1/2 (ERK1/2) by the EP4/PI3K pathway can also upregulate COX2 and mPGES-1 that enforce PGE₂ synthesis (Regan, 2003),

Figure 3

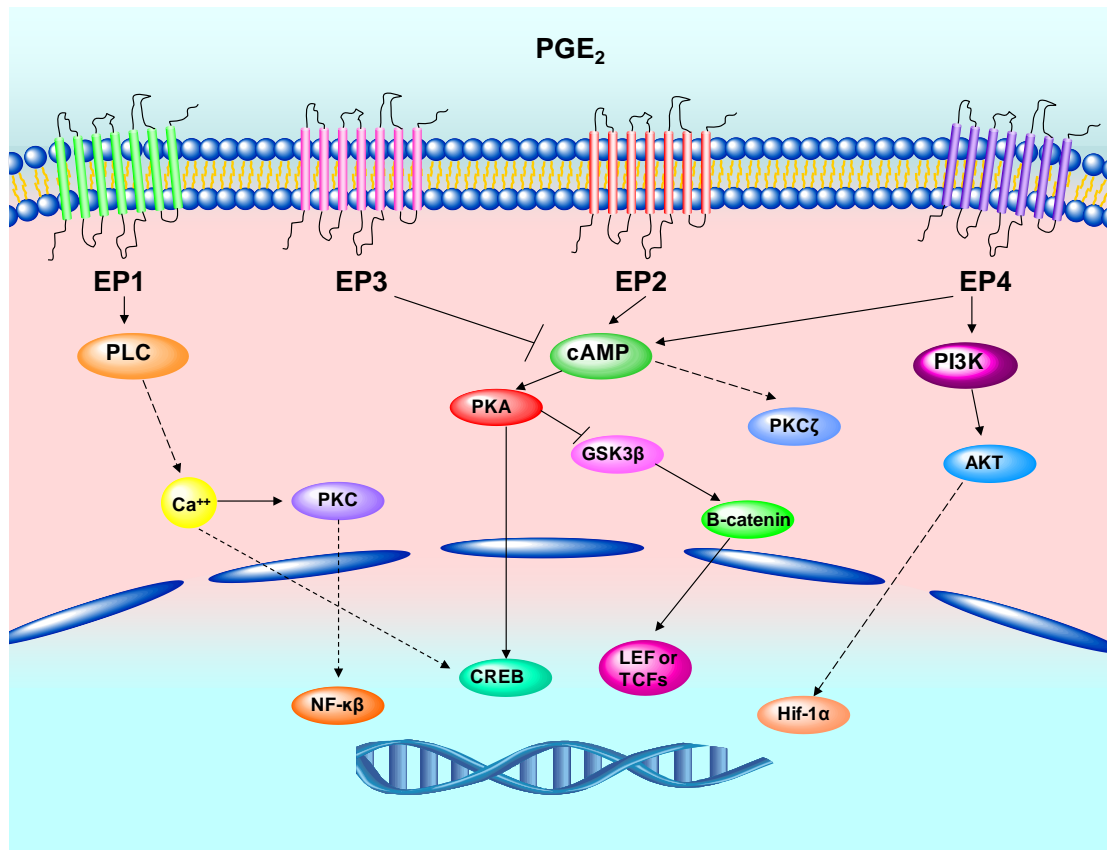


Figure 3. Schematic representation of EP receptor signaling.

PGE₂ signals through four different G-protein coupled receptors, EP1, EP2, EP3, and EP4. Signaling through the EP1 receptor is associated with activation of PLC and an intracellular calcium release. Signaling through the EP3 receptor is coupled with G_{ai} and inhibits production of cAMP. Signaling through the EP2 receptor is coupled with G_{as} and increases cAMP production. Signaling through the EP4 receptor can also increase cAMP, but is additionally associated with PI3K signaling. Shown are a few representative downstream signaling pathways and activated transcription factors associated with PGE₂ signaling.

suggesting that EP4 may increase autocrine production of PGE₂. It is clear that PGE₂ mediated effects on gene regulation and cellular responses result from the summation of signals and transcription factors generated downstream of the EP receptors, which depends on cell receptor repertoire, G-protein linkage, length of exposure to PGE₂ and signaling pathways selectively utilized by the cell.

The two main classes of leukotrienes, LTB₄, and the cysteinyl leukotrienes LTC₄, LTD₄ and LTE₄, interact with specific and independent GPCRs. LTB₄ interacts with its receptors BLT₁ and BLT₂, while the cysteinyl leukotrienes interact with the CysLT₁ and CysLT₂ receptors (Izumi et al., 2002). Two cannabinoid receptors, CB₁ expressed primarily in the central nervous system (CNS) and the peripheral CB₂ receptor have been identified (Croxford and Yamamura, 2005; Klein et al., 2003; Pertwee, 2006). The CB receptors, like the EP and leukotriene receptors, are highly conserved GPCRs. The signaling pathways downstream of the BLT, CysLT (Izumi et al., 2002; Massoumi and Sjolander, 2007; Thompson et al., 2008; Yokomizo et al., 2001) and CB (Hillard, 2000; Howlett, 2002; McAllister and Glass, 2002) receptors have been extensively explored in the context of inflammation, and specific downstream signaling is inherently dependent on cell type and G-protein linkage (Figure 4). Common features of signaling through the leukotriene receptors are activation of downstream pathways through Ca²⁺ mobilization and activation of PLC, and reduction in cAMP, which is opposite to that of PGE₂ signaling through EP2 and

EP4 receptors. In some ectopic expression systems, signaling through PKC, PI3K and/or MAPK have also been demonstrated.

Figure 4

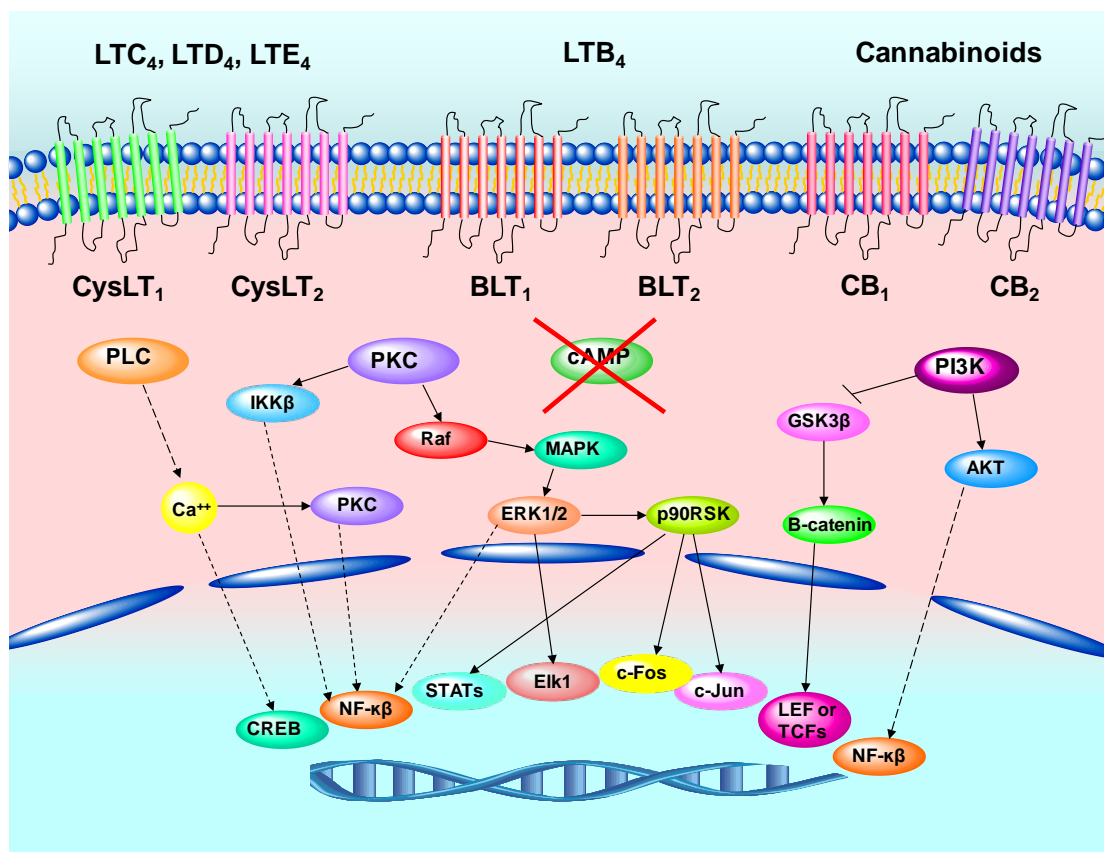


Figure 4. Schematic representation of leukotriene and endocannabinoid signaling.

The cysteinyl leukotrienes, LTC₄, LTD₄, and LTE₄ activate signaling through the CysLT₁ and CysLT₂ receptors. The leukotriene LTB₄ activates signaling through the BLT₁ and BLT₂ receptors. Cannabinoid signaling occurs through the CB₁ and CB₂ receptors. A simplified conglomerate representation of the downstream signaling events and activated transcription factors from these eicosanoid receptors is shown.

Prostaglandins and Hematopoiesis – Stimulatory and inhibitory functions:

Studies in the 1970s to 1990s documented regulatory roles for PGE₂ in hematopoiesis. Extensive work from the Pelus laboratory and others demonstrated a dose-dependent inhibition of the growth of human and mouse CFU-GM by PGE *in vitro* (Aglietta et al., 1980; Pelus et al., 1979; Pelus et al., 1980; Pelus et al., 1981; Taetle et al., 1980; Taetle and Koessler, 1980). Studies by Kurland, Pelus and colleagues also showed that PGE acted as a negative regulator of myeloid expansion to counterbalance positive signaling from the colony-stimulating factors (CSFs) in order to maintain appropriate HPC proliferation (Kurland et al., 1978; Kurland et al., 1979). PGE₂ was most selective for CFU-M and CFU-GM and was produced by macrophages in response to macrophage colony-stimulating factor (M-CSF) and GM-CSF, forming a selective feedback inhibition loop (Pelus et al., 1979). The role of PGE₂ in hematopoiesis and negative feedback regulation on myelopoiesis was documented by studies in mice differing in PGE synthetic capacity (Kincade et al., 1979; Kurland et al., 1979); demonstration of abnormal PGE₂ responses in leukemia patients (Aglietta et al., 1980; Moore et al., 1981; Pelus et al., 1980; Pelus et al., 1983; Taetle and Koessler, 1980); prognostic association of disordered PGE₂ response in MDS patients (Gold et al., 1983); abnormal HPC response in patients cured of germ cell tumors but progressing to acute leukemia (Leitner et al., 1987); and association of HPC response to PGE₂ with clinical response to Interferon-γ in chronic myelogenous leukemia (CML), chronic

lymphocytic leukemia (CLL), and Hodgkin's disease patients (Pelus and Vadhan-Raj, 1988; Vadhan-Raj et al., 1986). The effects of PGE₂ on myeloid HPC were considered to be direct, mimicked by dibutyryl-cAMP (db-cAMP) and blocked by agents preventing cAMP formation (Pelus, 1982). However, the Pelus lab later showed that PGE₂ could induce myeloid suppressor cells, particularly when administered *in vivo* (Gentile and Pelus, 1988; Pelus, 1989a; Pelus and Gentile, 1988), that had the phenotype F4/80⁺, Gr-1⁺, Mac-1⁺, consistent with myeloid-derived suppressor cells identified more recently (Luczynski et al., 2008; Parmiani et al., 2007).

Not all early studies demonstrated an inhibitory effect of PGE₂. Studies by Fehrer and Gidali in 1974 showed that short-term PGE₂ treatment of murine marrow cells *in vitro* increased day 9 CFU-S in cell cycle that was cAMP independent (Feher and Gidali, 1974). An increase in CFU-GM in S-phase was also seen after PGE₂ pulse exposure of human marrow (Verma et al., 1981). However, early studies evaluating *in vivo* dosing of PGE₂ in mice led to little or no increase in hematopoiesis (Gidali and Feher, 1977). In 1982, the Pelus lab showed that short-term exposure of human or mouse bone marrow to PGE₂ stimulated the production of cycling CFU-GM from a population of quiescent, non-cycling cells, probably stem cells that were dependent on time course and concentration of PGE₂ (Pelus, 1982) and were cAMP independent (Pelus, 1984). The Pelus lab also showed that the kinetics of PGE₂ exposure were critical for stimulatory versus inhibitory effects on HPC frequency and cell cycle (Pelus,

1984). In additional studies, it was shown that PGE₂ increased BFU-E and CFU-GEMM, which could be direct (Nocka et al., 1989) or mediated through factors released by T cells (Lu et al., 1986; Lu et al., 1987; Nocka et al., 1989). Recently, *in vitro* pulse exposure to PGE₂ was shown to increase the repopulating capacity of murine bone marrow cells and increase zebrafish kidney marrow recovery; however, mechanisms for increased engraftment and recovery were not determined (North et al., 2007).

The Pelus lab and others demonstrated that repetitive *in vivo* PGE₂ administration inhibits CFU-GM frequency and cell cycle rate, and decreases marrow and spleen cellularity (Gentile et al., 1983; Gentile and Pelus, 1987; Gentile and Pelus, 1988; Pelus and Gentile, 1988). These effects are both direct and mediated through accessory cells (Gentile and Pelus, 1988). Overall, previous work by our lab and others clearly demonstrate the importance of PGE₂ concentration, time course, and culture conditions in order to produce physiological effects. However, clonogenic HPC do not measure HSC function and we cannot rule out that effects observed on inhibition of HPC are in fact the result of PGE₂ signaling blocking differentiation and/or driving HSC towards self-renewal. In the transplant setting, particularly with cells manipulated *in vitro*, it is critical to define the roles of PGE₂ and other eicosanoids within the host bone marrow in order to determine whether modulation of PGE₂ or eicosanoid synthesis or action could further facilitate or potentially damage HSC engraftment. In Chapter 2, we present data that indicate treatment with PGE₂

results in a self-renewal division of HSC, and in Chapter 3, that blockade of PGE₂ synthesis with an NSAID or PGE₂ signaling with specific receptor antagonists can increase marrow and peripheral blood HPC.

The Plot Thickens – Leukotrienes and endocannabinoids:

Blockade of LTB₄ signaling pathways induces apoptosis and suppresses proliferation in neutrophils (Murray et al., 2003) and cancer cells (Ihara et al., 2007). The cysteinyl leukotrienes are important mediators in the pathophysiology of allergic conditions, particularly asthma (Braccioni et al., 2002; Kim and Luster, 2007). Three cysteinyl leukotriene receptor antagonists (pranlukast (Ultair™), montelukast (Singulair™), zafirlukast (Accolate™)) are FDA approved for use in asthma patients. Likewise, BLT₁ signaling can be blocked by a number of selective antagonists including U75302 and CP105696, and BLT₂ signaling is selectively antagonized by LY255283 (Kim and Luster, 2007). LTB₄ and cysteinyl leukotrienes are produced in the marrow microenvironment (Lindgren et al., 1993), stromal cell cultures and freshly isolated marrow mononuclear cells (Dupuis et al., 1997) and 5'-LOX is found in HPC (Bautz et al., 2001). LTB₄ is a potent granulocyte chemoattractant (Claesson et al., 1985; Mohle et al., 2001), while LTD₄ increases CD34⁺ and CD34⁺ CD38⁻ cell chemotaxis and transendothelial migration, which is blocked by a selective leukotriene receptor antagonist (Bautz et al., 2001). Accumulating evidence also suggests that leukotrienes play a role in regulation of hematopoiesis. While PGE₂ inhibits and COX inhibitors enhance HPC *in vitro*, LTB₄, LTC₄ and LTD₄ increase mouse

and human HPC (Braccioni et al., 2002; Elsas et al., 2008; Vore et al., 1989), which is inhibited by 5'-LOX inhibitors (Braccioni et al., 2002; Kozubik et al., 1994; Vore et al., 1989). Moreover, PGE₂ stimulates (Lu et al., 1987; Rossi et al., 1980), while LTB₄ and LTC₄, inhibit and LOX inhibitors enhance (Estrov et al., 1988) early and late erythroid progenitor cells. In mice, dual COX inhibition enhances HPC recovery (Kozubik et al., 1994), while selective 5'-LOX inhibitors decrease CFU-GM and blast colony-forming cells (CFU-BL) (Vore et al., 1989), suggesting an effect on a cell earlier than the CMP and CLP. The addition of LTB₄ to UCB cells cultured with growth factors enhances HPC proliferation with concomitant reduction in total CD34⁺ cells. The selective LTB₄ receptor antagonist CP105696 enhances production of CD34⁺ cells and blocks HPC proliferation (Chung et al., 2005). Overall, the available data suggest that LTB₄ signaling decreases HSC self-renewal and increases differentiation, while blocking LTB₄ receptors increases self-renewal and blocks differentiation. Thus, the use of a leukotriene receptor antagonist or LOX inhibitor in the post-transplant setting may favor self-renewal. In addition, since blockade of COX makes more arachidonic acid available to the LOX pathway (Bertolini et al., 2002; Elsas et al., 2008), the use of a COX inhibitor post-transplant could promote HSC differentiation via leukotrienes, which is discussed further in Chapter 5.

The cannabinoids have been implicated in positive and negative effects on mature cells of the immune system (Croxford and Yamamura, 2005; Klein et al.,

2000); however, little is known about their effects on earlier hematopoietic cells. Anandamide can act as a synergistic growth factor for HPC (Valk et al., 1997) and has an apoptotic effect on erythrocytes (Bentzen and Lang, 2007). The endocannabinoid 2-AG also stimulates proliferation of HPC (Valk and Delwel, 1998) and hematopoietic cells expressing the CB₂ receptor migrate in response to ligation by 2-AG (Jorda et al., 2002). Recently, 2-AG has been shown to increase CFU-GEMM colony formation and cell migration (Patinkin et al., 2008), and activation of CB receptors on murine ESC by cannabinoids promotes hematopoietic differentiation (Jiang et al., 2007). Additionally, Non-Hodgkin's lymphoma cells have abnormally high levels of CB₂ receptor expression (Rayman et al., 2007), suggesting a potential proliferative role for cannabinoids. It is clear that prostaglandins, leukotrienes and endocannabinoids may have vital roles in hematopoietic homeostasis, and evaluating their responses is critical to understanding eicosanoid function and development of eicosanoid-based therapeutic strategies, which is the focus of this thesis dissertation.

The Yin and Yang of Eicosanoid Regulation:

In many physiological systems, prostaglandins, leukotrienes and endocannabinoids exhibit compensatory or opposing roles (reviewed in Table 1). The action of many NSAIDs, which block the production of PGE₂, may also act by increasing signaling via endocannabinoids (Fowler, 2004). PGE₂-glycerol has been shown to mobilize calcium, activate signal transduction pathways (Nirodi et al., 2004) and have neurological effects opposite of those induced by 2-AG

(Sang et al., 2007). Furthermore, prostaglandins and leukotrienes have been shown to have numerous opposing roles in pulmonary fibrosis (Huang and Peters-Golden, 2008) and in other systems they may act in a coordinate fashion (Guerrero et al., 2008). It is particularly noteworthy that cannabinoid ligands block CXCR4 signaling (Coopman et al., 2007; Ghosh et al., 2006) and neutrophil migration (Kurihara et al., 2006; Nilsson et al., 2006), which one would expect to negatively affect homing, but enhance spontaneous release from marrow that may facilitate mobilization. In contrast, PGE₂ enhances CXCR4 expression and signaling (Goichberg et al., 2006; Salcedo et al., 2003) that might facilitate homing, but inhibit migration, which could dampen mobilization. In Chapter 3, we highlight studies where we have shown that inhibition of PGE₂ biosynthesis *in vivo* in mice and baboons, with NSAIDs, enhances spontaneous as well as G-CSF-induced mobilization, and agonism of cannabinoid receptors results in similar levels of enhanced HPC mobilization.

Table 1

| System Affected | Cannabinoids | Leukotrienes | Prostaglandins |
|----------------------------|---|---|---|
| CXCR4/CXCL12 | ↓ (Ghosh et al., 2006; Coopman et al., 2007) | -- | ↑ (Goichberg et al., 2006; Salcedo et al., 2003) |
| cAMP | ↓ (Klein et al., 2003; Pertwee, 2006) | ↓ (Izumi et al., 2002) | ↑↓ (George et al., 2007) |
| MMP-9 | ↑ (Rosch et al., 2006) | ↑ (Ichiyama et al., 2007) | ↓ (Reno et al., 2004) |
| Neutrophil migration | ↓ (Kurihara et al., 2006; Nilsson et al., 2006) | ↑ (Guerrero et al., 2008) | ↑ (Desouza et al., 2005) |
| Inflammation | ↓ (Croxford and Yamamura, 2005; Klein et al., 2003) | ↑ (Kim and Luster, 2007; Braccioni et al., 2002) | ↑ (Lazarus, 2006; Narumiya, 2003) |
| Apoptosis | ↑ (Lombard et al., 2007; Powles et al., 2005) | ↓ (Ihara et al., 2007) | ↓ (George et al., 2007) |
| Hematologic Cell Migration | ↑ (Jorda et al., 2002; Kishimoto et al., 2005) | ↑ (Bautz et al., 2001) | ↑↓ (Legler et al., 2006; Panzer and Uguccioni, 2004) |
| Myelopoiesis | ↑ (Valk and Delwel, 1998; Valk et al., 1997) | ↑ (Claesson et al., 1985; Stenke et al., 1991; Stenke et al., 1993) | ↓ (Pelus et al., 1979; Pelus et al., 1980; Pelus et al., 1981; Pelus, 1982) |
| Erythropoiesis | ↓ (Bentzen and Lang, 2007) | ↓ (Estrov et al., 1988) | ↑ (Rossi et al., 1980; Lu et al., 1984; Lu et al., 1986; Lu et al., 1987) |

Table 1. Opposing roles of cannabinoids, leukotrienes and prostaglandins.

Shown is a summary of a few highlighted physiological processes that are regulated by the eicosanoid system. In most cases, cannabinoids act in an opposing role to prostaglandin, while leukotrienes regulate similarly or opposing functions to prostaglandin. The dual prostaglandin effects shown for some physiological processes are the result of EP receptor subtype differences.

Chapter 2. Prostaglandin E₂ Enhances Hematopoietic Stem Cell Homing, Survival and Proliferation

Introduction:

Hematopoietic stem cell transplantation with bone marrow, mobilized peripheral blood, or UCB, is a proven therapy for malignant and nonmalignant hematologic diseases and metabolic disorders. Repopulation of hematopoiesis is a multi-step process that can be adversely affected by the inability of HSC to migrate/home to appropriate marrow niches or poor engrafting efficiency and self-renewal. Insight into the intrinsic and extrinsic mechanisms regulating these critical functions can lead to new strategies to improve HSC transplantation efficacy.

As previously mentioned, PGE₂ is the most abundant eicosanoid and a mediator of numerous physiological systems (Miller, 2006). There is considerable evidence that demonstrates regulatory roles for PGE₂ in hematopoiesis. PGE₂ dose-dependently inhibits growth of human and mouse CFU-GM *in vitro* (Pelus et al., 1979; Pelus et al., 1981) and myelopoiesis *in vivo* (Gentile et al., 1983) but stimulates erythroid and multilineage progenitor cells (Lu et al., 1984; Lu et al., 1987). Short-term *ex vivo* treatment of marrow cells with PGE₂ increases the proportion of mouse CFU-S (Feher and Gidali, 1974) and human CFU-GM (Verma et al., 1981) in cell cycle. In addition, PGE₂ can stimulate production of cycling human CFU-GM from a population of quiescent

cells, possibly stem cells, which is critically dependent on timing, duration of exposure and concentration (Pelus, 1982). Recently, it was shown that pulse exposure to PGE₂ *ex vivo* increased HSC frequency of murine bone marrow cells and enhanced kidney marrow recovery in zebrafish (North et al., 2007). However, while it is clear that PGE₂ can affect HSC and HPC, the mechanisms of action of PGE₂ on hematopoietic function have yet to be determined.

In this Chapter, we show that PGE₂ acts directly on murine HSC to enhance their frequency and facilitates engraftment. PGE₂ also provides a competitive advantage that is maintained during multiple serial transplantations, with full multi-lineage reconstitution. The enhanced chimerism observed throughout long-term serial transplantation, however, is not due to sustained competitive advantage, but rather is the result of increased HSC numbers produced by initial exposure to PGE₂ preceding/during the primary transplant. Enhanced HSC engraftment induced by PGE₂ results from increased homing of HSC, mediated through up-regulation of the chemokine receptor CXCR4, implicated in HSC homing (Lapidot et al., 2005), and selective stimulation of primitive HSC survival and self-renewal associated with up-regulation of the inhibitor of apoptosis protein Survivin that is required for HSC maintenance and entry into cell cycle (Fukuda et al., 2002; Fukuda and Pelus, 2001). Our studies describe novel mechanisms for enhancement of HSC function by PGE₂ and support a translational strategy to facilitate HSC transplantation.

Materials and Methods:

Mice and human cord blood

C57Bl/6 (CD45.2) mice were purchased from Jackson Laboratories (Bar Harbor, ME). B6.SJL-PtcrAPep3B/BoyJ (BOYJ) (CD45.1), C57Bl/6 X BOYJ-F1 (CD45.1/CD45.2) and NOD.Cg-*Prkdcscid IL2rgtm1Wjl/Sz* (NSG) mice were bred in-house. Mice used in transplant studies received Doxycycline feed for 30 days post-transplant. The Institutional Animal Care and Use Committee (IACUC) of Indiana University School of Medicine (IUSM) approved all protocols. Human UCB was obtained from Wishard Hospital, Indianapolis, IN with Institutional Review Board (IRB) approval.

Flow cytometry

All antibodies were purchased from BD Biosciences unless otherwise noted. For detection and sorting of KL and SKL cells, streptavidin conjugated with PE-Cy7 (to develop biotinylated MACS[®] lineage antibodies (Miltenyi Biotech, Auburn, CA)), c-kit-APC, Sca-1-PE or APC-Cy7, CD45.1-PE, CD45.2-FITC, and CD34-PE were used. For SLAM SKL, we utilized Sca-1-PE-Cy7, c-kit-FITC, CD150-APC (eBiosciences, San Diego, CA), CD48-biotin (eBiosciences) and streptavidin-PE. UCB CD34⁺ cells were detected using anti-human-CD34-APC. For multi-lineage analysis, APC-Cy7-Mac-1, PE-Cy7-B-220 and APC-CD3 were used. EP receptors were detected with rabbit anti-EP(1-4) antibodies (Cayman Chemicals, Ann Arbor, MI) and FITC-goat-anti-rabbit IgG (Southern Biotech,

Birmingham, AL). Expression of CXCR4 was analyzed using streptavidin-PECy7, c-kit-APC, Sca-1-APC-Cy7, and CXCR4-PE. Apoptosis was measured with FITC-Annexin-V or FITC-anti-active caspase-3. For Survivin and active caspase-3 detection, cells were permeabilized and fixed using the CytoFix/CytoPerm kit (BD) and stained with anti-active-caspase-3-FITC Flow Kit (BD) or Survivin-PE (R&D Systems, Minneapolis, MN). For cell cycle analysis, cells were stained with Hoechst-33342 (Molecular Probes, Eugene, OR) and Pyronin-Y (Sigma Aldrich, St. Louis, MO) or FITC bromodeoxyuridine (BrdU) Flow Kit (BD). Analyses were performed on an LSRII and sorting on either a FACS Aria or FACS Vantage sorter (BD).

dmPGE₂ pulse-exposure

16,16-dimethyl Prostaglandin E₂ (dmPGE₂) in methyl acetate (Cayman Chemicals) was evaporated on ice under nitrogen gas, reconstituted in 100% ethanol (ETOH) at a final concentration of 0.1 M and stored at -20 °C. For pulse exposure, cells were incubated with dmPGE₂ diluted in media, on ice, for 2 hours, with gentle vortexing every 30 minutes. After incubation, cells were washed twice in media at 4 °C before use. Vehicle-treated cells were processed in an identical manner, using the equivalent ETOH concentration.

Limiting dilution competitive and non-competitive transplantation

Whole bone marrow (WBM) cells (CD45.2) were treated on ice for 2 hours with 1 μM dmPGE₂ (Cayman) or 1x10⁻³ % ETOH per 1x10⁶ cells in phosphate

buffered saline (PBS). After incubation, cells were washed twice and mixed with 2×10^5 congenic CD45.1 competitor marrow cells at various ratios and transplanted intravenously into lethally-irradiated (1100-cGy split dose) CD45.1 mice. Peripheral blood (PB) CD45.1 and CD45.2 cells were determined monthly by flow cytometry. For head-to-head competitive analysis, WBM from CD45.1 and CD45.2 mice were treated with vehicle or dmPGE₂ and mixed with 2×10^5 competitor marrow cells from CD45.1/CD45.2 mice at various ratios and transplanted into lethally-irradiated CD45.1/CD45.2 mice. The proportion of CD45.1, CD45.2, and CD45.1/CD45.2 cells in PB was determined monthly. HSC frequency was quantitated by Poisson statistics using L-CALC software (Stem Cell Technologies, Vancouver BC, Canada) with <5% contribution to chimerism considered negative. Competitive repopulating units (CRU) were calculated as described (Harrison, 1980). For secondary transplants, 2×10^6 WBM from previously transplanted CD45.1/CD45.2 mice at a 1:1 ratio at 20 weeks post-transplant were injected into lethally-irradiated CD45.1/CD45.2 mice in non-competitive fashion. Tertiary, quaternary, and quinary transplants were performed in a similar manner.

Long-term competitiveness assay

Whole BM cells from CD45.1 and CD45.2 donors were acquired, and treated with either vehicle or dmPGE₂ as described. One cohort of lethally irradiated CD45.1/CD45.2 mice were transplanted with 5×10^5 vehicle treated CD45.1 cells, and 5×10^5 dmPGE₂ treated CD45.2 cells. A separate cohort was

transplanted in a similar fashion with strain and treatment groups switched. After 12 weeks, PB chimerism was evaluated and bone marrow was acquired and antibody stained for CD45.1/CD45.2 and SLAM SKL markers. CD45.1 and CD45.2 SLAM SKL cells were sorted, and a second group of lethally irradiated CD45.1/CD45.2 mice were transplanted with 2.5×10^2 CD45.1 SLAM SKL, 2.5×10^2 CD45.2 SLAM SKL, and 2.0×10^5 CD45.1/CD45.2 WBM competitors. Contribution to PB chimerism was evaluated 12 weeks later.

Analysis of HSC homing

Whole BM from CD45.2 mice was labeled with 5-(and -6)-carboxyfluorescein diacetate succinimidyl ester (CFSE) (Molecular Probes) washed and treated with either 1 μ M dmPGE₂ or vehicle and 2×10^7 cells transplanted into lethally-irradiated CD45.2 mice. After 16 hours, femurs and tibias were flushed, lineage positive cells depleted using MACS[®] microbeads and Lin^{neg} cells stained for SKL and the total number of CFSE⁺ WBM, KL and SKL cells determined. For congenic homing studies, Lin^{neg} CD45.1 cells were treated with 1 μ M dmPGE₂, vehicle, or PBS and 2×10^6 cells transplanted into lethally-irradiated CD45.2 mice. After 16 hours, CD45.1 SKL cells in recipient BM were quantitated. For competitive homing studies, Lin^{neg} cells from CD45.2 and CD45.1 mice were FACS sorted, treated with dmPGE₂ or vehicle and 3×10^4 CD45.1 (vehicle or dmPGE₂ treated) plus 3×10^4 CD45.2 (dmPGE₂ or vehicle treated) SKL cells transplanted into lethally-irradiated CD45.1/CD45.2 mice. To evaluate the role of CXCR4 in homing, Lin^{neg} CD45.2 cells were treated with

vehicle or 1 μM dmPGE₂ plus 10 μM AMD3100 (AnorMed Inc., Vancouver, BC, Canada), 2×10^6 treated cells injected into lethally-irradiated CD45.1 mice and homed SKL cells analyzed 16 hours post-transplant. Homing of human UCB cells was evaluated in NSG mice. UCB mononuclear cells were isolated on Ficoll-PaqueTM Plus (Amersham Biosciences, Piscataway, NJ), treated with either dmPGE₂ or vehicle, and 4×10^7 cells transplanted into each of 5 sublethally-irradiated (250 cGy) mice. Homed CD34⁺ cells were analyzed 16 hours post-transplant.

Expression of EP receptors, CXCR4 and Survivin

Lin^{neg} marrow cells were stained for SKL, SLAM or CD34, each of the 4 EP receptors and surface receptor expression on KL, SKL and SLAM SKL and CD34^{neg} SKL cells determined by FACS. For human EP receptors, UCB CD34⁺ cells were positively selected with MACS[®] microbeads (Fukuda and Pelus, 2001), stained for CD34 and CD38 and each of the EP receptors and surface receptor expression determined by FACS. To evaluate CXCR4, Survivin and active caspase-3, Lin^{neg} cells or CD34⁺ UCB were treated on ice with either 1 μM dmPGE₂ or vehicle control for 2 hours, washed, and then cultured in RPMI-1640/10% heat inactivated-fetal bovine serum (HI-FBS) at 37 °C for 24 hours, stained for SKL (murine cells) and CXCR4, Survivin, and/or active caspase-3 as described earlier, and analyzed by FACS.

Migration assays

Chemotaxis to SDF-1 α was determined using a two-chamber Costar Transwell ((Cambridge, MA), 6.5 mm diameter, 5 μ m pore) as previously described (Fukuda and Pelus, 2006). Briefly, dmPGE₂ and vehicle-treated Lin^{neg} bone marrow cells were cultured in RPMI/10% HI-FBS overnight to allow for up-regulation of CXCR4, washed, resuspended at 2x10⁶ cells per ml in RPMI/0.5% BSA and 0.1 ml added to the top chamber of the transwells, with or without rmSDF-1 α (R&D Systems) in the bottom and/or top chamber, and incubated for 4 hours at 37 °C. Cells completely migrating to the bottom chamber were enumerated by flow cytometry. Percent migration was calculated by dividing total cells migrated to the lower well by the cell input multiplied by 100. Migration of SKL cells was determined by comparison of the proportion of SKL cells in input and migrated populations. For UCB migration, CD34⁺ cells were MACS[®] selected as described and migration assays performed as described for mouse, using rhSDF-1 α (R&D Systems).

Cell cycle analysis

For *in vitro* cell cycle analysis, Lin^{neg} cells were treated with either 1 μ M dmPGE₂ or vehicle and cultured in StemSpan[®] - Serum Free Media (Stem Cell Technologies) with rmSCF (50 ng/ml) (R&D Systems), rhFlt-3 and rh-thrombopoietin (TPO) (100 ng/ml each) (Immunex, Seattle, WA). After 20 hours, cells were stained for SLAM SKL, fixed, permeabilized and stained with Hoechst-

33342 followed by Pyronin-Y. The proportion of SLAM SKL cells in G₀, G₁, S and G₂/M phase was determined by FACS quantitation of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). For *in vivo* cell cycle analysis, CD45.2 mice were lethally-irradiated and transplanted with 5x10⁶ dmPGE₂ or vehicle-treated Lin^{neg} CD45.1 cells. Recipient mice received 1 mg/ml BrdU (Sigma) in drinking water and 1 mg/mouse BrdU by intraperitoneal (IP) injection. After 16 hours, recipient marrow was isolated, lineage depleted, stained for CD45.1, SKL and BrdU and the proportion of homed CD45.1⁺ SKL cells that were BrdU⁺ determined by FACS.

Apoptosis assays

Lin^{neg} cells were treated with 1 μM dmPGE₂ or vehicle, and incubated in RPMI/2% HI-FBS at 37 °C without growth factors. After 24 hours, the cells were stained for SLAM SKL and Annexin-V or active caspase-3 and the proportion of apoptotic cells determined by FACS. For dose ranging studies, cells were cultured as described using a dose range of 0.1 nM to 1 μM dmPGE₂ or vehicle control.

Quantitative-RT-PCR

Total RNA was obtained using the absolute RNA purification kit (Stratagene, La Jolla, CA). A constant amount of RNA was reverse transcribed with random primers (Promega, Madison, WI) and MMLV-reverse transcriptase (Promega) as described (Fukuda and Pelus, 2001). DNase and RNase free

water (Ambion, Austin, TX) was added to obtain a final concentration equivalent of 10 ng RNA/ μ l and 5 μ l used for QRT-PCR. Primers for SYBR Green QRT-PCR were designed to produce an amplicon size of 75-150 base pairs.

Sequences of primers are listed in Table 2. QRT-PCR was performed using Platinum SYBR Green qPCR supermix UDG with ROX (Invitrogen, Carlsbad, CA) in an ABI-7000 (Applied Biosystems, Carlsbad, CA) or MxPro-3000 (Agilent, LaJolla, CA). Dissociation curves were determined on each analysis to confirm that only one product was obtained.

Statistical analysis

All pooled values are expressed as Mean \pm Standard Error of the Mean (SEM). Statistical differences were determined using the paired or unpaired two-tailed t-test function in Microsoft Excel as appropriate.

Table 2

| Gene | Location | F/R | Primer Sequence |
|----------|--------------|-----|------------------------------------|
| EP1 | Human (946) | F | 5' - TTGGCGGCTCTCGGA - 3' |
| | Human (1071) | R | 5' - GCCACCAACACCAGCATT- 3' |
| | Mouse (942) | F | 5' - GCTCCTTGCGGCATTAGTGTG- 3' |
| | Mouse (1038) | R | 5' - TGCGGTCTTTCGGAATCGT- 3' |
| EP2 | Human (899) | F | 5' - AGGAGAGGGGAAAGGGTGTC - 3' |
| | Human (1000) | R | 5' - AATCGTGAAAGGCAAGGAGC - 3' |
| | Mouse (1028) | F | 5' - CGTTATCCTCAACCTCATTCGC - 3' |
| | Mouse (1156) | R | 5' - TCCGTCTCCTCTGCCATCG - 3' |
| EP3 | Human (1053) | F | 5' - CCGCATCACGACCGAGAC - 3' |
| | Human (1146) | R | 5' - AATCGTGAAAGGCAAGGAGC - 3' |
| | Mouse (772) | F | 5' - TTGCTGGCTCTGGTGGTGAC - 3' |
| | Mouse (868) | R | 5' - GCTGGACTGCGAGACGGC - 3' |
| EP4 | Human (612) | F | 5' - ATTCGTCCGCCTCCTTGA - 3' |
| | Human (705) | R | 5' - GCCACCAGGTGGCCCA - 3' |
| | Mouse (1476) | F | 5' - TGACCCAAGCAGACACCACCT - 3' |
| | Mouse (1586) | R | 5' - TCCCACTAACCTCATCCACCAA - 3' |
| CXCR4 | Human (58) | F | 5' - TACACCGAGGAAATGGGGT - 3' |
| | Human (172) | R | 5' - CAGTTAGAAGATGATGGAGTAGATGG 3' |
| | Mouse (502) | F | 5' - CTCGCTATTGTCCACGCCAC - 3' |
| | Mouse (651) | R | 5' - CCCTGACTGATGTCCCCCTG - 3' |
| SURVIVIN | Human (212) | F | 5' - GACGACCCCATAGAGGAAC - 3' |
| | Human (347) | R | 5' - CCTTTGCAATTTTGTTCTTG - 3' |
| | Mouse (321) | F | 5' - GAGAGCCAAGAACAAAATTGC - 3' |
| | Mouse (435) | R | 5' - CTCAGCATTAGGCAGCCAG - 3' |
| HPRT | Human (429) | F | 5' - GCAGACTTTGCTTTCCTTGG - 3' |
| | Human (484) | R | 5' - TTGCGACCTTGACCATCTTT - 3' |
| | Mouse (224) | F | 5' - TTGCTGACCTGCTGGATTAC - 3' |
| | Mouse (342) | R | 5' - TATGTCCCCCGTTGACTGA - 3' |

The numbers in parentheses indicate the location of the first base of the primer for the gene of interest. Forward (F) and reverse (R) primers used are indicated as shown.

Results:

PGE₂ increases LTR-HSC frequency and engraftment

It was previously shown that PGE₂ stimulates proliferation, cycling and differentiation of quiescent bone marrow cells into colony forming cells, suggesting that PGE₂ enhances HSC function (Pelus, 1982). However, hematopoietic repopulation in myeloablated hosts is the only true measure of HSC function (Harrison, 1980). A recent report showed that pulse exposure to dmPGE₂ enhanced HSC frequency when transplanted into irradiated mice (North et al., 2007). We have confirmed enhancement of HSC frequency by PGE₂. In addition, using a limiting-dilution, competitive head-to-head transplant model of CD45.2 and CD45.1 congenic grafts in CD45.1/CD45.2 hybrid mice that permits quantitative comparison of engraftment and competitiveness of HSC from control and dmPGE₂ treatment groups, as well as endogenous repopulation of host cells within the same animal, we now show that short-term dmPGE₂ exposure produces stable long-term enhancement of HSC frequency and engraftment upon serial transplantation and that short-term exposure to dmPGE₂ increases the number of CRU and stably enhances HSC competitiveness (Figure 5A). At 12 weeks post-transplant, analysis of PB showed significantly increased chimerism of dmPGE₂ treated cells compared to vehicle treated cells, with ~4-fold increase in HSC frequency and CRU, quantitative measures of long-term-repopulating capacity (Figure 5B).

Figure 5

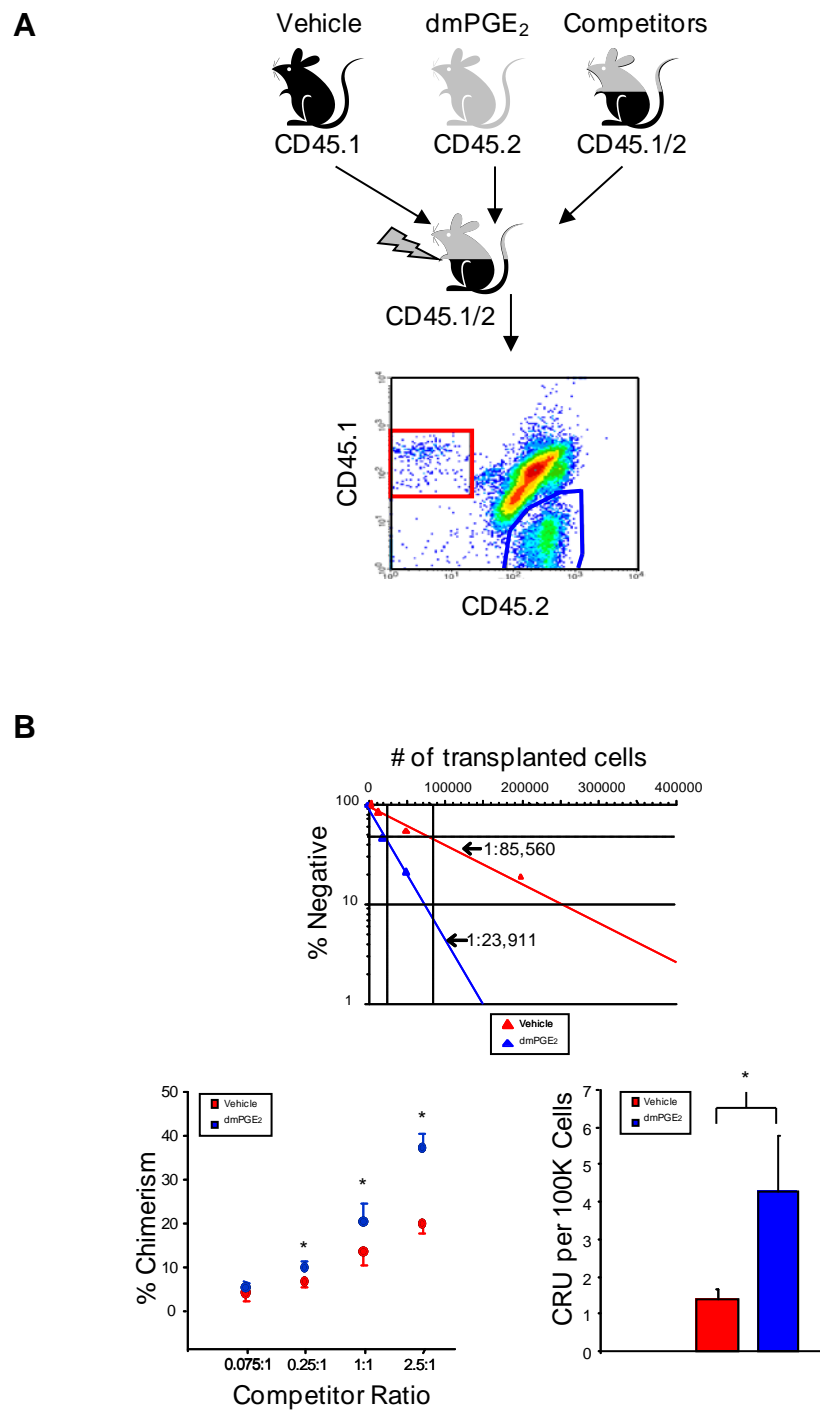


Figure 5. PGE₂ enhances hematopoietic stem cell engraftment.

(A) Test bone marrow from CD45.1 or CD45.2 mice were treated with vehicle or dmPGE₂ respectively. CD45.1/CD45.2 hybrid marrow cells were used as competitors. Limiting dilutions were transplanted into lethally irradiated (1100cGys, split dose) CD45.1/CD45.2 hybrid mice and chimerism in PB analyzed for 20 weeks. A representative flow plot detecting each cell population is shown.

(B) Frequency analysis (top) for vehicle (red) or dmPGE₂ (blue) pulsed cells, determined by Poisson statistics, at 12 weeks; $P_0 = 85,560$ (vehicle) and $P_0 = 23,911$ (dmPGE₂ treated). Chimerism in PB and CRU analysis is shown at 12 weeks (Mean \pm SEM). Data represent 2 pooled experiments, N=5 mice per group, per experiment, each assayed individually.

* $P < 0.05$ compared to vehicle control.

Throughout 20 weeks post-transplant, an ~4-fold increase in HSC frequency was maintained, indicating that the effect of dmPGE₂ pulse-exposure was stable (Table 3). At 32 weeks post-transplant, reconstitution was seen for peripheral B- and T-lymphoid and myeloid lineages, with no discernible differences in lineage contribution between untreated competitor cells, dmPGE₂ or vehicle treated cells (Figure 6).

Marrow was harvested from primary transplanted animals at 20 weeks post-transplant and transplanted into secondary recipients (Figure 7) to validate expansion and self-renewal of LTRC previously exposed to dmPGE₂ and vehicle. Analysis of PB 12 and 24 weeks after secondary transplant showed multi-lineage reconstitution by cells from all transplanted mice, clearly demonstrating the self-renewal of primary transplanted LTRC. Unlike the primary transplant, multi-lineage reconstitution by dmPGE₂ treated cells showed an elevated myeloid lineage reconstitution, though this was not seen in subsequent transplants. The increase in chimerism resulting from dmPGE₂ exposure seen in primary donors was also observed in secondary transplants without any additional treatments. Upon further serial transplant, vehicle treated HSC could only be detected at low level after tertiary transplant, suggesting a loss of HSC self-renewal, likely an effect of low HSC number. In contrast, HSC were maintained at a higher level for PGE₂ treated HSC through quaternary transplant, suggesting higher HSC numbers and/or self-renewal.

Table 3

| Weeks Post- transplant | Repopulating Cell Frequency | | |
|---------------------------------------|------------------------------------|--------------------------|--------------------------|
| | Vehicle | dmPGE₂ | Fold Increase |
| 4 | 1:69,466 | 1:16,619 | 4.18 |
| 8 | 1:85,560 | 1:24,613 | 3.48 |
| 12 | 1:85,560 | 1:23,911 | 3.58 |
| 16 | 1:85,560 | 1:23,911 | 3.58 |
| 20 | 1:89,586 | 1:21,753 | 4.12 |

Table 3. PGE₂ pulsed grafts have increased LTRC.

HSC frequency analysis in recipients of vehicle or dmPGE₂ treated bone marrow over 20 weeks. Fold change indicates increase in frequency of engraftment of dmPGE₂ pulsed cells compared to vehicle.

Figure 6

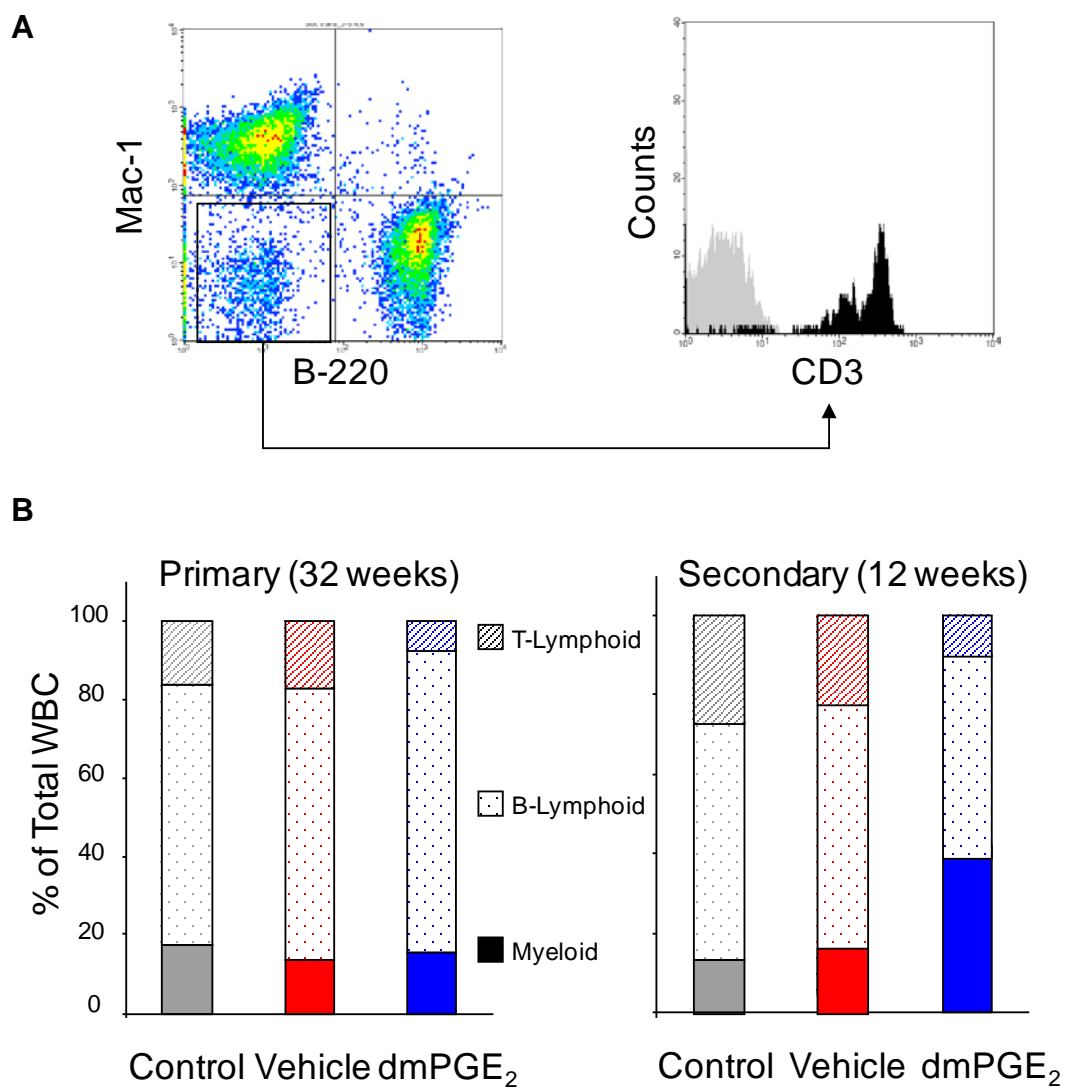


Figure 6. Full multi-lineage reconstitution after transplantation with PGE₂ pulsed grafts.

(A) Representative FACS plots of multi-lineage reconstitution showing Mac-1⁺ cells (myeloid), B220⁺ cells (B-Lymphoid), or CD3⁺ cells (T-Lymphoid) from peripheral blood of a hybrid CD45.1/CD45.2 mouse transplanted with vehicle or dmPGE₂ pulsed CD45.1 or CD45.2 bone marrow cells, respectively.

(B) Relative contribution to lineages of myeloid (M), B (B) and T-lymphoid (T). Multi-lineage analysis for primary transplant (32 weeks) and a cohort of 5 mice that received transplants from primary transplanted mice at 20 weeks, with analysis 12 weeks later. For primary transplanted mice at 32 weeks, vehicle treated cells were (Mean \pm SEM) 14.1 \pm 3.5% M, 70.8 \pm 1.1% B, and 17.8 \pm 1.4% T, versus dmPGE₂ treated cells which were 15.7 \pm 2.5% M, 76.9 \pm 3.4% B, and 7.5 \pm 1.2% T. For secondary transplanted mice at 12 weeks, vehicle treated cells were 15.7 \pm 5.3% M, 60.3 \pm 4.8% B, and 22.1 \pm 3.6% T, versus dmPGE₂ treated cells which were 37.0 \pm 6.5% M, 52.3 \pm 5.4% B, and 9.0 \pm 1.4% T.

* P<0.05 versus vehicle control.

Figure 7

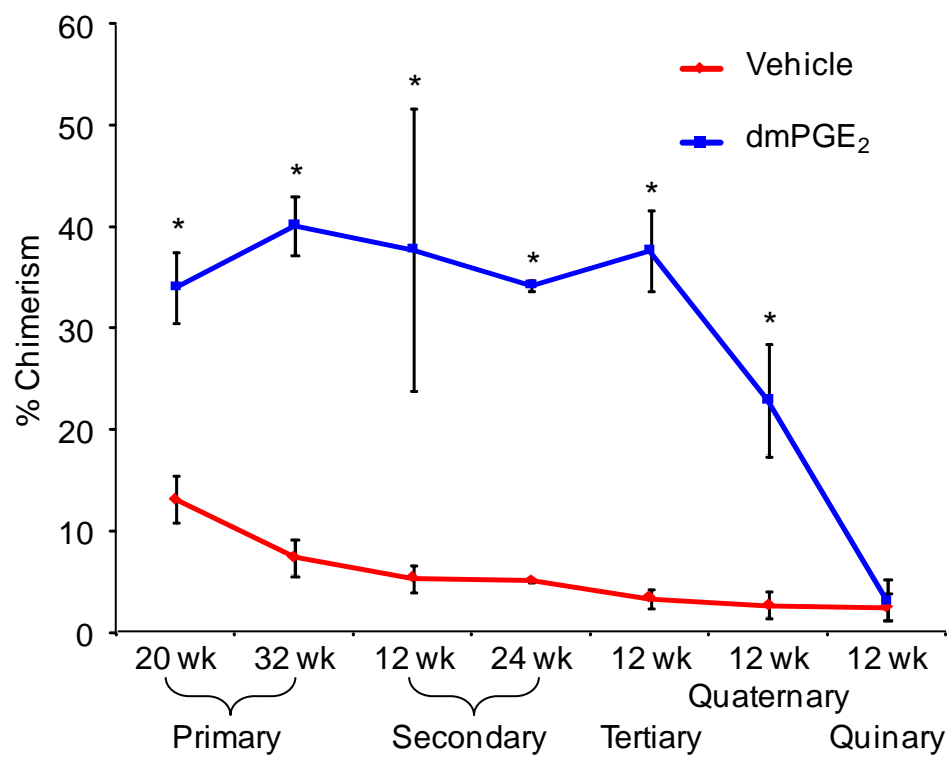


Figure 7. PGE₂ pulsed grafts maintain repopulating ability through serial transplantations.

Increased chimerism of dmPGE₂ treated cells versus vehicle is shown for primary transplant at 20 weeks (time of secondary transplant) and in a sub-cohort at 32 weeks (time of 12 week analysis of secondary transplant), for secondary transplant at 12 weeks and 24 weeks, and for tertiary, quaternary, and quinary at 12 weeks. Data for 20 week primary transplant were from 2 pooled experiments, N=5 mice per group, per experiment, each assayed individually. Data for secondary, tertiary, quaternary and quinary transplants were from N=5 mice per group, each assayed individually. Data are expressed as Mean ± SEM.

*P<0.05.

Lack of inherent enhanced competitiveness of dmPGE₂ pulsed grafts in secondary transplants

Increased chimerism of dmPGE₂ pulsed grafts through multiple serial transplants in head-to-head competition with vehicle pulsed grafts could simply be the result of a higher number of HSCs homing and engrafting in the primary graft, taking available niche space and self-renewing; or could be the result of epigenetic changes in HSC elicited by the dmPGE₂ treatment (Araki et al., 2009; Chung et al., 2009); dmPGE₂ preferentially enhancing a subset of HSC with enhanced inherent competitiveness; or an unknown mechanism eliciting a long-term competitive advantage. If serial transplant results are simply due to an increase in HSC homing and engraftment of the primary graft, then “equalizing” the HSC content from dmPGE₂ pulsed grafts and vehicle pulsed grafts should result in equal repopulation in secondary recipients. To test this hypothesis, dmPGE₂ pulsed and vehicle pulsed WBM were transplanted head-to-head into lethally irradiated hybrid mice, and chimerism was evaluated 12 weeks later (Figure 8A). As was seen in earlier transplants, dmPGE₂ resulted in significantly increased chimerism compared to vehicle treated control cells (Figure 8B). Bone marrow from these primary recipient mice was harvested and FACS sorted for SLAM SKL cells, and equal numbers of the sorted SLAM SKL cells were transplanted head-to-head into secondary recipients along with radioprotecting WBM competitors, with no additional treatment. Equalizing the HSC content in the secondary grafts resulted in no differential increase in repopulating ability in

secondary recipients. This suggests that the enhanced engraftment of dmPGE₂ treated HSC in primary recipients is a function of increased numbers of homed HSC and their subsequent self-renewal.

Figure 8

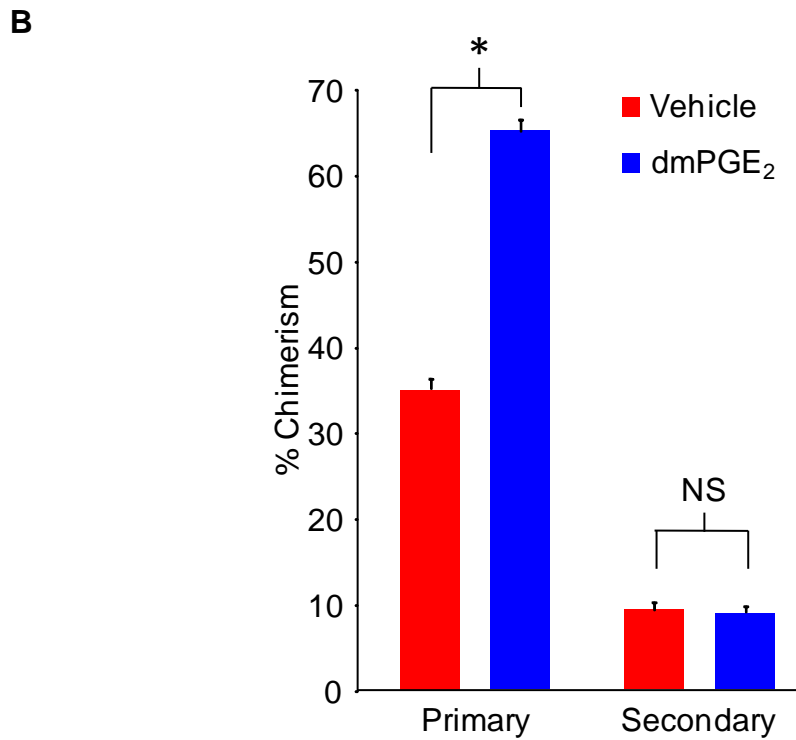
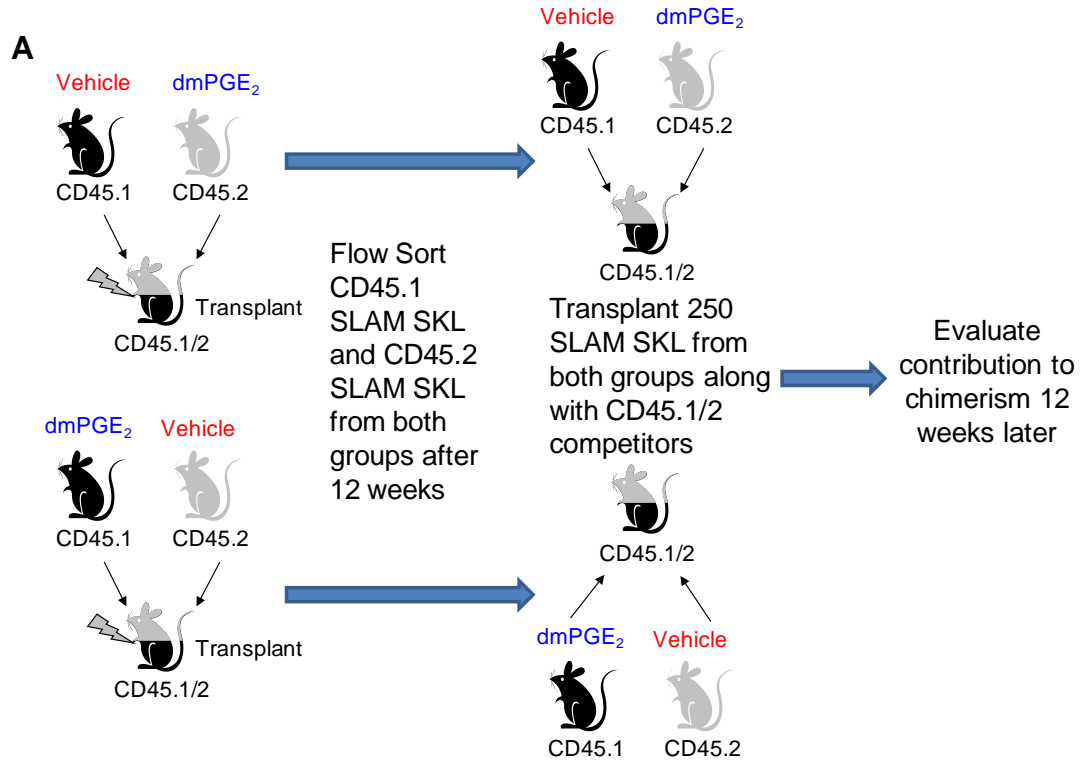


Figure 8. dmPGE₂ pulsed HSC do not have an inherent competitive advantage in secondary transplants.

(A) Schematic representation of experimental design. Whole bone marrow from CD45.1 mice and CD45.2 mice was treated with both vehicle and dmPGE₂ and then transplanted head-to-head into lethally irradiated (1100cGys, split dose) CD45.1/CD45.2 hybrid mice as shown (2.5×10^5 cells per group). Chimerism was analyzed at 12 weeks, and bone marrow from recipients was collected, stained with fluorescent antibodies for phenotypic markers, and cells sorted for SLAM SKL. SLAM SKL cells were transplanted head-to-head into a second cohort of lethally irradiated CD45.1/CD45.2 mice along with 2.0×10^5 WBM CD45.1/CD45.2 competitors, and chimerism analyzed 12 weeks later.

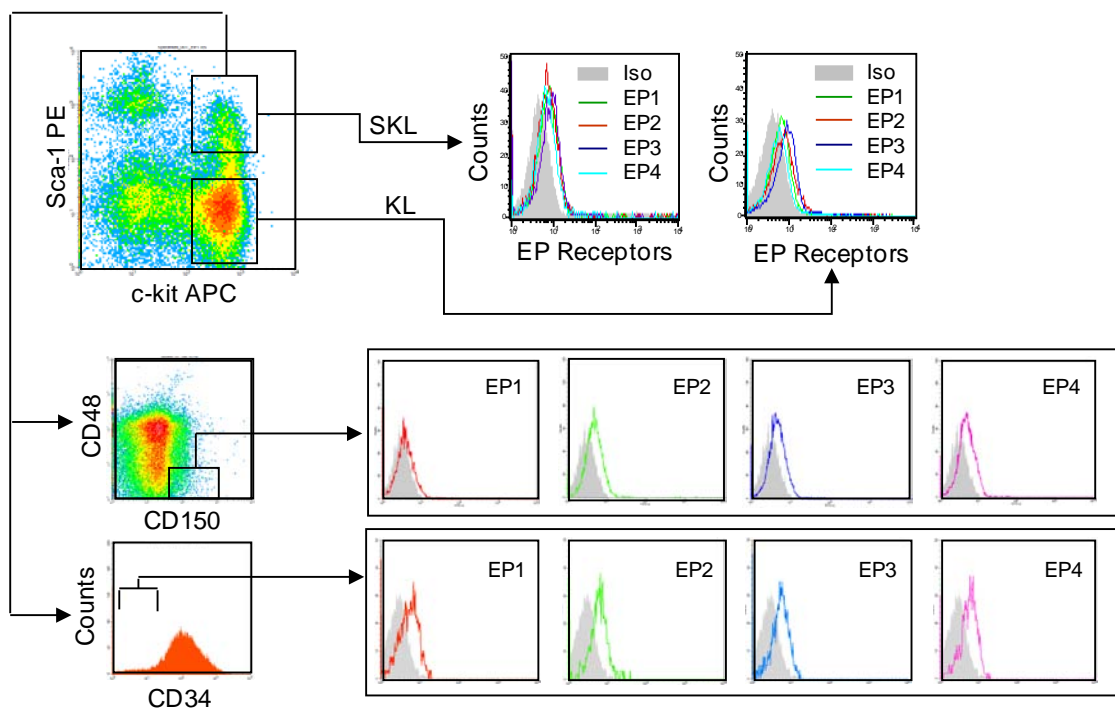
(B) Chimerism in PB is shown for 12 weeks after the primary transplant, and 12 weeks after the secondary transplant (Mean \pm SEM). N=10 mice per group (total of 20 mice for primary and 20 mice for secondary); * $P < 0.001$.

Murine and human HSC and HPC express PGE₂ receptors

PGE₂ interacts with 4 specific, highly conserved G-protein coupled receptors; EP1-EP4 (Sugimoto and Narumiya, 2007), with EP receptor repertoire accounting for multiple, sometimes opposing responses attributed to PGE₂ (Breyer et al., 2001). While EP receptor expression has been observed in dendritic cells (Harizi et al., 2003), monocytes (Panzer and Uguccioni, 2004), and early zebrafish hematopoietic tissue (Villablanca et al., 2007), EP receptor expression on hematopoietic stem and progenitor cell populations is not known. Analysis of EP receptors on KL cells, enriched for hematopoietic progenitor cells, SKL cells enriched for multipotent progenitor cells as well as HSC, and SLAM SKL and CD34⁻ SKL cells, highly enriched for primitive repopulating HSC (Chen et al., 2008; Kiel et al., 2005), showed that all four EP receptors are expressed on these hematopoietic cell populations (Figure 9A). Analogous to murine cells, all four receptors are expressed on human CD34⁺ UCB cells enriched for HSC and CD34⁺ CD38⁻ cells that contain the most primitive human HSC (Figure 9B). Quantitative RT-PCR showed that mRNA for all four EP receptors is detected in the whole bone marrow cell population and in FACS sorted KL, SKL and primitive CD34⁻ SKL cells (Figure 10A) and on CLP (Lin^{neg} c-kit^{low} Sca-1^{low} IL7R⁺) and CMP (Lin^{neg} c-kit⁺ Sca-1⁻ CD34⁺) progenitor cells (not shown). Similarly, QRT-PCR analysis detected mRNA for all four EP receptors in purified CD34⁺ and CD34⁺ CD38⁻ UCB cells (Figure 10B).

Figure 9

A



B

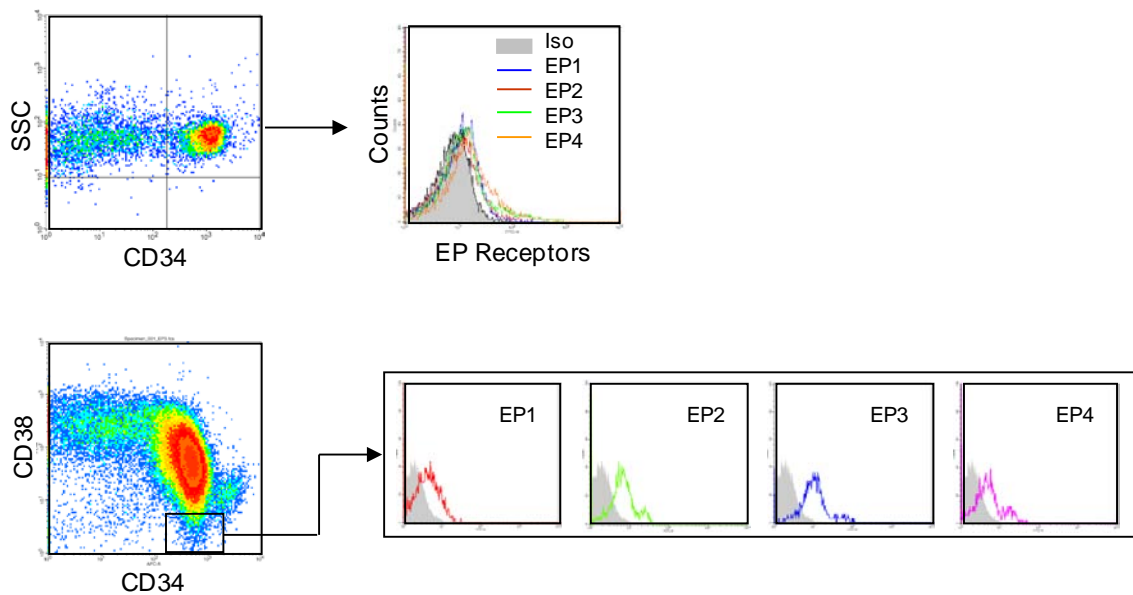


Figure 9. EP receptors are expressed on murine and human HSC and HPC.

(A) Representative FACS gating of Lin^{neg} murine bone marrow showing c-kit⁺ and Sca-1⁺ gates and SLAM (CD150⁺ CD48⁻) and CD34 gating of SKL cells.

EP1-EP4 surface receptor expression on murine KL, SKL, SLAM SKL and CD34⁻ SKL cells is shown.

(B) Representative FACS gating of human CD34⁺ and CD34⁺ CD38⁻ UCB cells.

EP surface receptor expression on CD34⁺ and CD34⁺ CD38⁻ cells is shown.

Figure 10

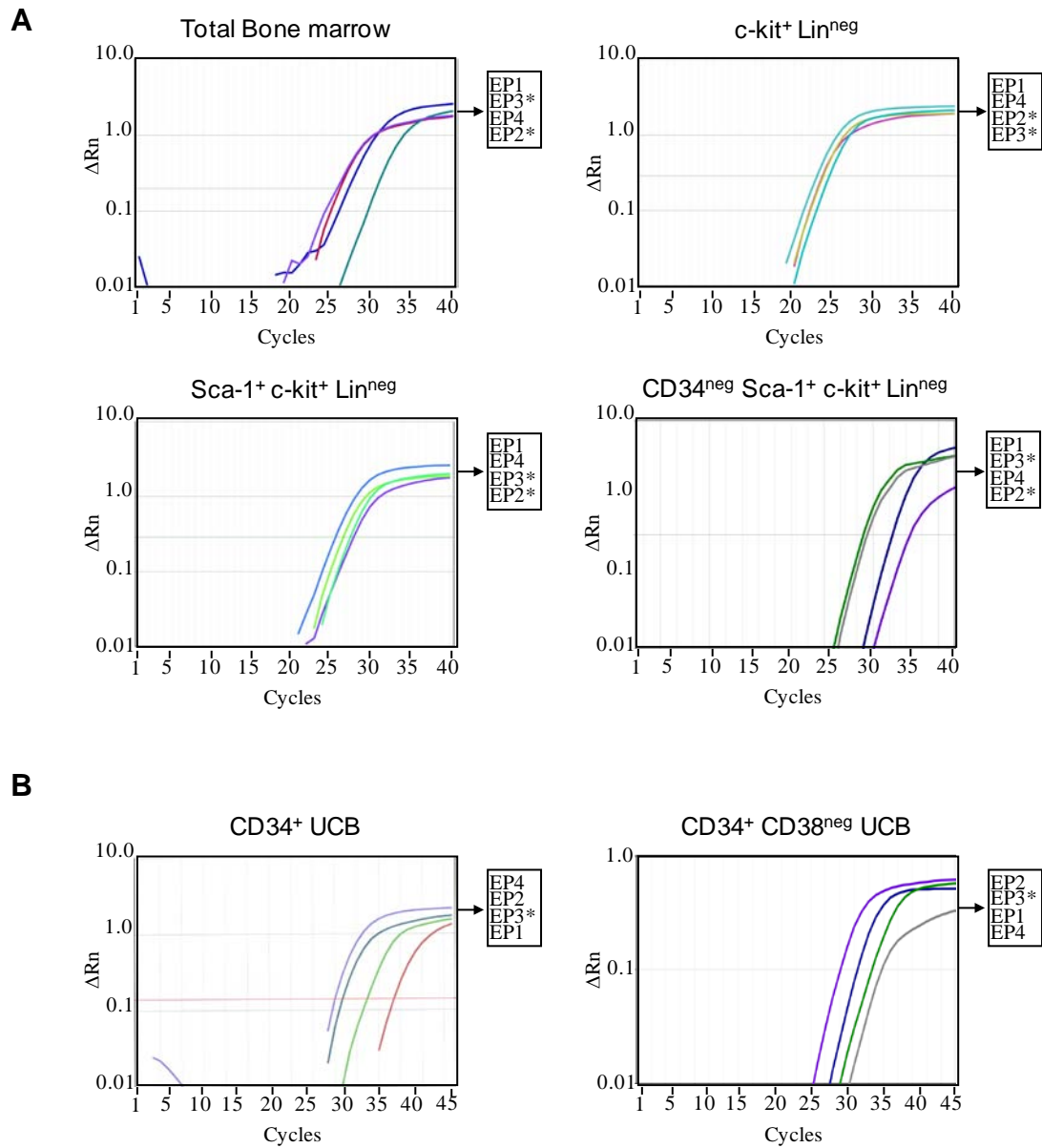


Figure 10. Amplification plots of mRNA for PGE₂ receptors.

(A) Primers designed to specifically detect murine EP1, EP2, EP3 or EP4 were used for QRT-PCR (with SYBR green) and plots with an activation step of 50 °C for 2 minutes, denaturation at 95 °C for 2 minutes and amplification for 45 cycles at 95 °C-15 seconds, 50 °C-30 seconds, 72 °C-30 seconds are shown. Plots corresponding to specific EP receptors are indicated in each amplification plot, where the legend key on the right shows the relative order of transcripts top to bottom. * Denotes the presence of at least 2 dissociation peaks indicating the presence of splice variants.

(B) EP receptor amplification on human UCB CD34⁺ and CD34⁺ CD38⁻ cells with the same QRT-PCR procedure as above.

PGE₂ increases HSC homing efficiency

Enhanced HSC engraftment by PGE₂ could result from increased HSC number and/or cell cycle status (Ramshaw et al., 1995), effects on facilitating cells (Gandy et al., 1999) or effects on HSC homing or proliferation in the host marrow (Lapidot et al., 2005). In order to evaluate the mechanism of action of PGE₂ on HSC engraftment, we first utilized CFSE labeled dmPGE₂ or vehicle treated WBM cells transplanted into lethally-irradiated hosts to assess HSC homing. Total CFSE⁺ cells homing to bone marrow as well as the number of homed events within the KL and SKL cell populations were quantitated (Figure 11A). No difference in the percentage of CFSE⁺ cells homing to marrow was observed between dmPGE₂ and vehicle treated cells when total WBM cells were evaluated; however, significantly more SKL cells homed to the marrow compared to control. In a congenic model, where homed cells are detected based upon CD45 cell surface variants, a significantly greater percentage of dmPGE₂ treated SKL cells homed to marrow (Figure 11B) compared to vehicle treated or to non-manipulated control cells. No difference in homing efficiency was seen between untreated and vehicle treated cells.

To determine whether the enhancing effect of dmPGE₂ on HSC homing was direct or indirect, we compared homing of highly purified SKL cells from both CD45.2 and CD45.1 mice in a head-to-head model. FACS sorted SKL cells were treated with dmPGE₂ or vehicle and transplanted into lethally irradiated

Figure 11

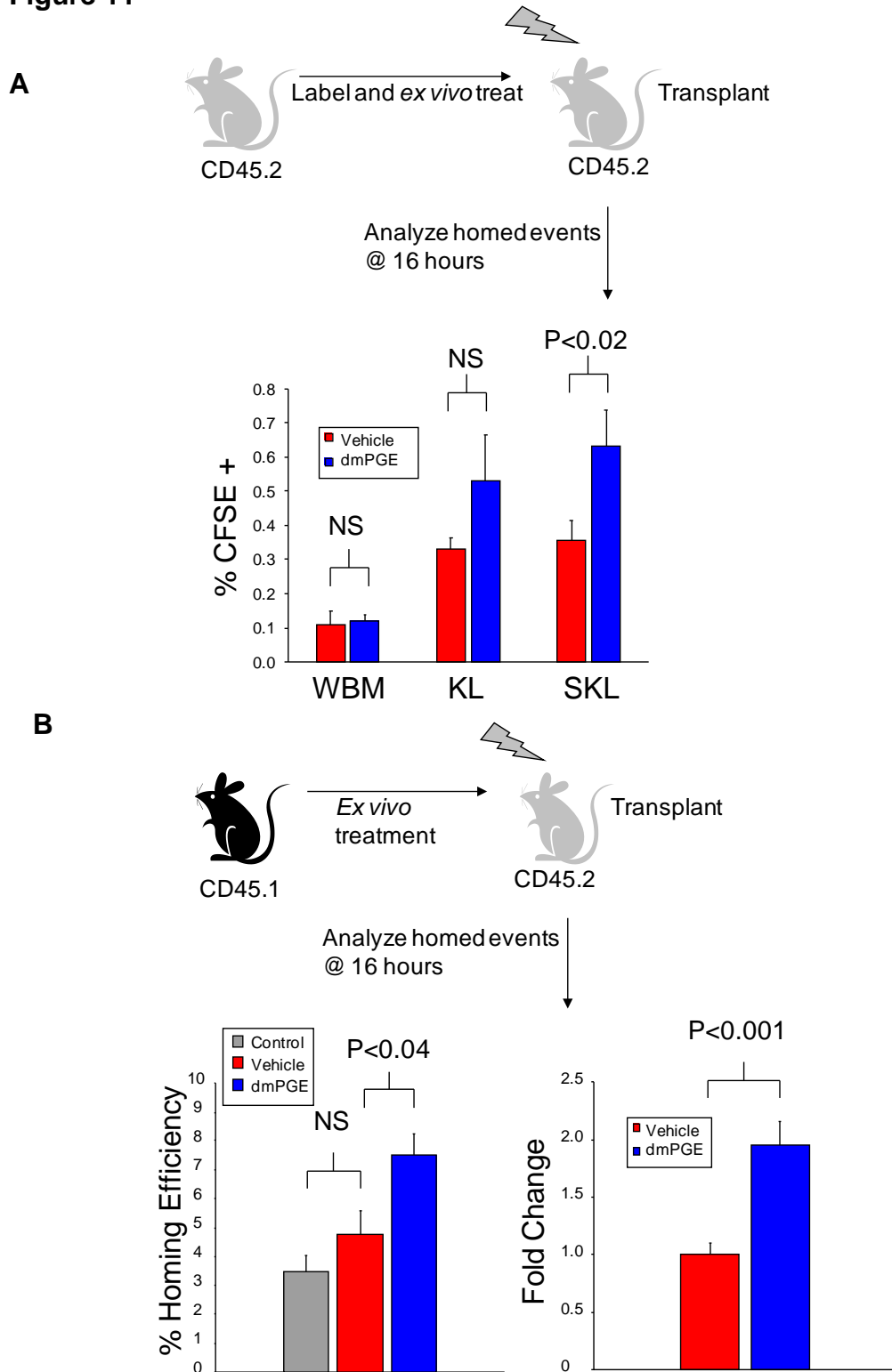


Figure 11. PGE₂ increases homing efficiency of HSC.

(A) Test murine bone marrow cells were labeled with CFSE and treated with vehicle (red) or dmPGE₂ (blue) and 2×10^7 labeled and treated WBM cells were transplanted into lethally irradiated mice. Sixteen hours later, bone marrow was analyzed by FACS for homed events. Data are expressed as Mean \pm SEM, N=6 mice per group, each assayed individually.

(B) Test bone marrow cells from CD45.1 mice were treated with PBS, vehicle or dmPGE₂, and 2×10^7 treated WBM cells were transplanted into lethally irradiated CD45.2 mice. Sixteen hours later bone marrow was analyzed for homed SKL cells. The left panel shows representative data from 1 experiment, N=3 mice per group, each assayed individually. The right panel shows the combined increase in homing efficiency of SKL cells after dmPGE₂ treatment for 3 experiments (N=6 mice per group, per experiment, each assayed individually).

CD45.1/CD45.2 mice. An additional cohort was transplanted with congenic strain and treatment groups switched to test for strain bias. Similar to studies using WBM, dmPGE₂ pulse-exposure of purified SKL cells increased their homing efficiency by 2-fold (Figure 12), strongly suggesting a direct effect on HSC. Although SKL cells are not a homogenous HSC population, they are highly enriched for LTRC (Okada et al., 1992). Immunodeficient mice offer the ability to evaluate human HSC function in an *in vivo* setting (Dick et al., 1992) and are a validated model for human HSC homing (Jetmore et al., 2002). To verify that the enhancing effect of dmPGE₂ on mouse HSC homing is also seen on human HSC, UCB mononuclear cells were pulsed with dmPGE₂ or vehicle and HSC homing evaluated in sublethally-irradiated NSG mice (Figure 13). Similar to mouse HSC, dmPGE₂ pulse-exposure significantly enhanced the homing efficiency of UCB CD34⁺ cells.

PGE₂ increases HSC CXCR4 and chemotaxis to SDF-1 α

The SDF-1 α /CXCR4 axis is believed to play a major role in HSC and HPC trafficking and chemoattraction/homing to the bone marrow microenvironment (Lapidot et al., 2005). In addition, up-regulation of CXCR4 on human CD34⁺ cells (Goichberg et al., 2006) and endothelial cells (Salcedo et al., 2003) by PGE₂ has been reported, and PGE₂ can increase monocyte chemotaxis to SDF-1 α (Panzer and Uguccioni, 2004). We therefore evaluated whether the mechanism of improved homing of dmPGE₂ treated HSC and HPC resulted from up-regulation of SDF-1 α /CXCR4 signaling. Pulse-exposure to dmPGE₂

Figure 12

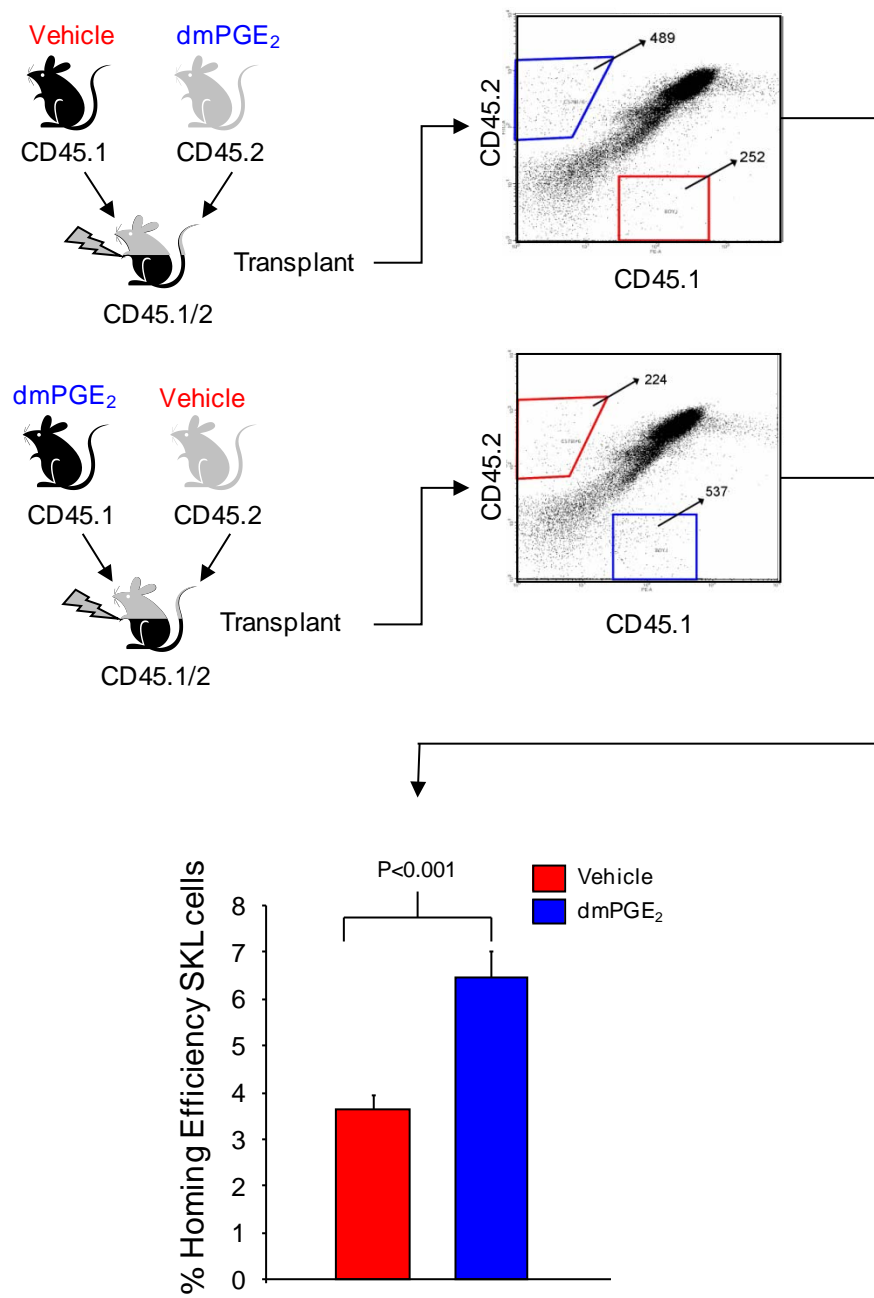


Figure 12. Competitive head-to-head increase in HSC homing.

SKL cells from CD45.1 and CD45.2 mice were isolated by FACS sorting and treated with either dmPGE₂ or vehicle. Five lethally irradiated CD45.1/CD45.2 hybrid mice received 3x10⁴ vehicle treated CD45.1 sorted SKL plus 3x10⁴ dmPGE₂ treated CD45.2 SKL cells (top panel). Five mice received a similar transplant with treatment groups switched between strains (bottom panel). Representative flow gating of marrow 16 hours post-transplant and combined data for the homing efficiency of dmPGE₂ or vehicle treated, sorted SKL cells (Mean ± SEM, N=10 mice, each assayed individually) are shown.

Figure 13

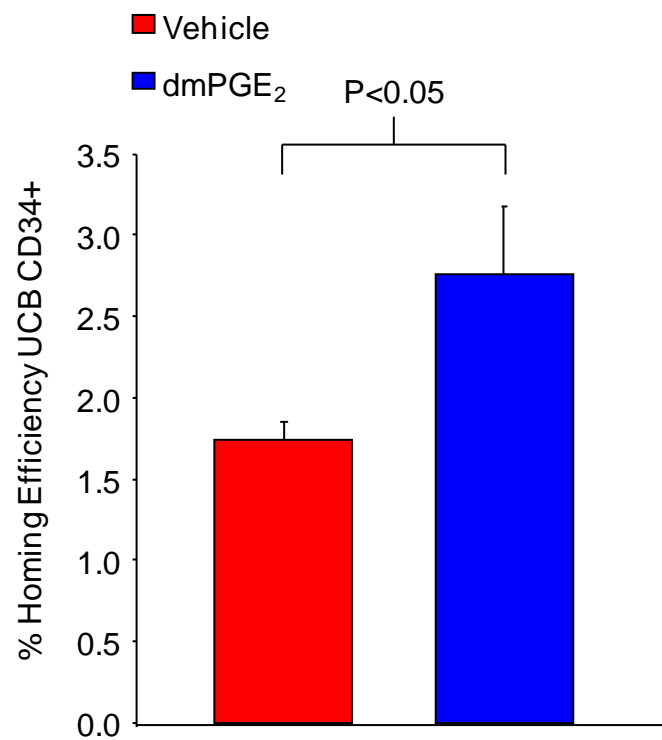


Figure 13. PGE₂ increases homing efficiency of UCB CD34⁺ cells in NSG mice.

Low density mononuclear cells (LDMC) from UCB were isolated and treated with either dmPGE₂ or vehicle. Five sublethally irradiated NSG mice received dmPGE₂ treated LDMC and 5 received vehicle treated LDMC. Bone marrow was analyzed 16 hours later and the number of CD34⁺ cells determined and homing efficiency calculated. Data are Mean ± SEM for N=5 mice, each assayed individually.

Figure 14

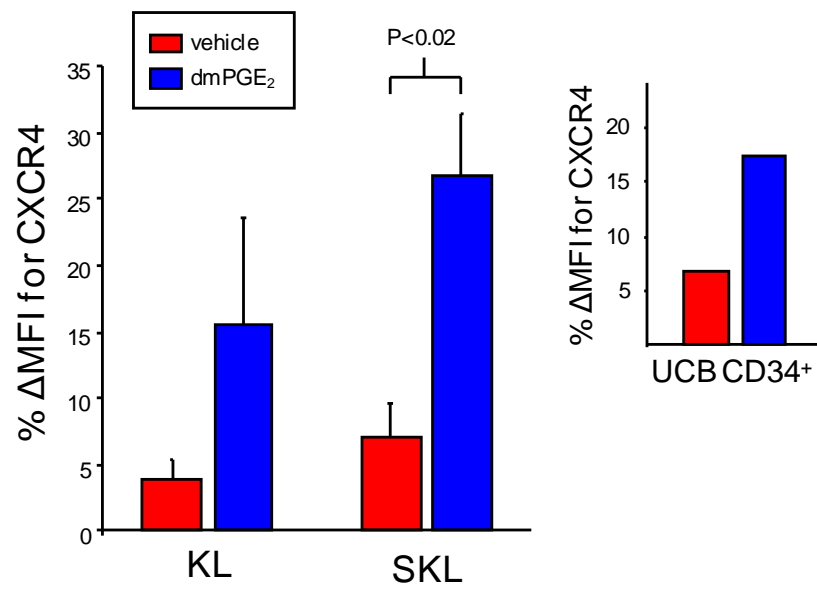


Figure 14. CXCR4 receptor expression is increased on murine and human HSC and HPC after dmPGE₂ treatment.

CXCR4 expression (Mean \pm SEM; N=3) on murine KL and SKL cells, and human UCB CD34⁺ cells 24 hours after treatment with dmPGE₂. Data are expressed as percent change in mean fluorescence intensity (MFI) of CXCR4 due to treatment with dmPGE₂ or vehicle.

increased CXCR4 expression on KL and SKL cells (Figure 14). Similarly, dmPGE₂ increased CXCR4 expression on CD34⁺ UCB cells. QRT-PCR demonstrated 2.65-fold elevated CXCR4 mRNA levels in dmPGE₂ treated SKL cells compared to vehicle.

In vitro, HSC and HPC selectively migrate to a gradient of SDF-1 α (Kim and Broxmeyer, 1998), a process that is believed to reflect their marrow homing capacity. We evaluated the effect of dmPGE₂ treatment on HSC chemotaxis to a gradient of SDF-1 α in *in vitro* transwell migration assays to determine if PGE₂-mediated CXCR4 up-regulation enhanced chemotaxis. Both vehicle and dmPGE₂ treated SKL cells demonstrated significant migration to 1-1000 ng/ml SDF-1 α , however, chemotaxis was significantly higher in cells treated with dmPGE₂ (Figure 15). Analysis of positive and negative gradients indicated that the dmPGE₂-enhancing effect on SKL cell chemotaxis did not result from a nonspecific increase in chemokinesis (Figure 15-top inset). Enhanced migration to SDF-1 α by dmPGE₂ was also observed using FACS-sorted SKL cells, suggesting a direct effect on HSC (Figure 15-bottom inset). Chemotaxis of UCB CD34⁺ cells to SDF-1 α was also significantly enhanced by pulse-exposure to dmPGE₂ and migration was blocked by the selective CXCR4 antagonist AMD3100 (Hatse et al., 2002), indicating a specific effect mediated through the CXCR4 receptor (Figure 16A).

To specifically determine if up-regulated CXCR4 played a role in the enhanced homing observed after dmPGE₂ treatment, cells were treated with

Figure 15

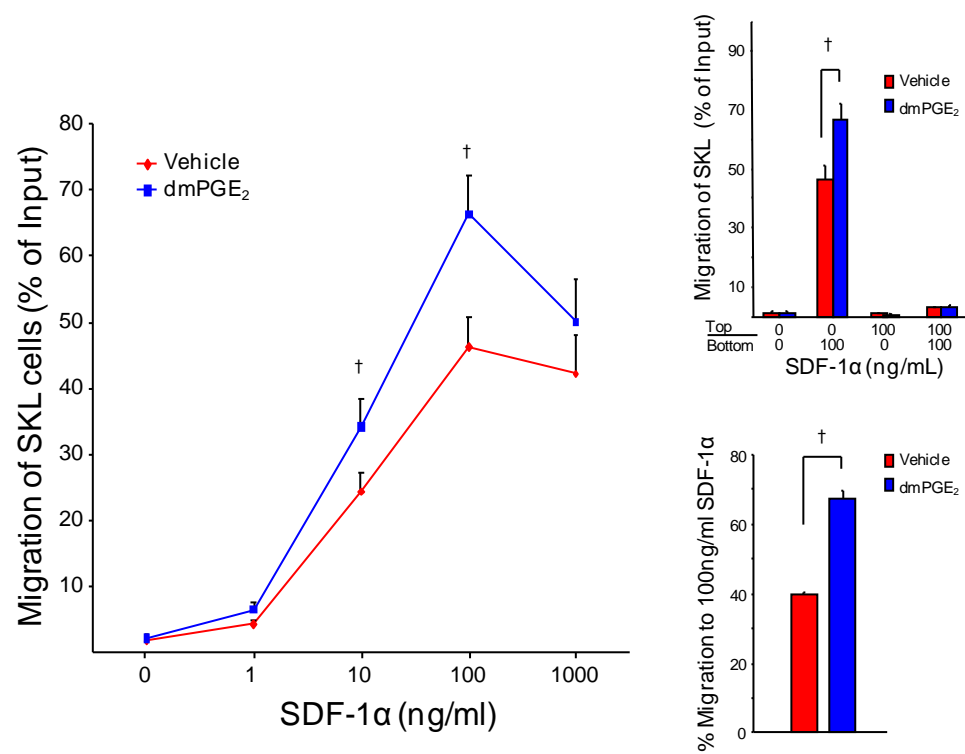


Figure 15. Increase in HSC and HPC migration to SDF-1 α after dmPGE₂ treatment.

Freshly isolated Lin^{neg} cells were pulsed with dmPGE₂ or vehicle for 2 hours, washed and resuspended in media with 10% HI-FBS and cultured at 37 °C for 16 hours. After incubation, cells were washed, resuspended in RPMI/0.5% BSA and allowed to migrate to rmSDF-1 α for 4 hours. Total cell migration was quantitated by flow cytometry. Data are the Mean \pm SEM percent migration for 3 experiments. [†] P<0.05 for dmPGE₂ treated cells compared to cells treated with vehicle.

(Top Inset) Percent migration of gated SKL cells to positive (100 ng/ml SDF-1 α in bottom chamber), negative (100 ng/ml SDF-1 α in upper chamber) or neutral (100 ng/ml SDF-1 α in both upper and bottom chambers) gradients. Data are the Mean \pm SEM percentage migration for 3 experiments. [†] P<0.05 for dmPGE₂ treated cells compared to cells treated with vehicle.

(Bottom Inset) Percent migration of sorted SKL cells to 100 ng/ml SDF-1 α . Data are the Mean \pm SEM percentage migration for 3 experiments. [†] P<0.05 for dmPGE₂ treated cells compared to cells treated with vehicle.

Figure 16

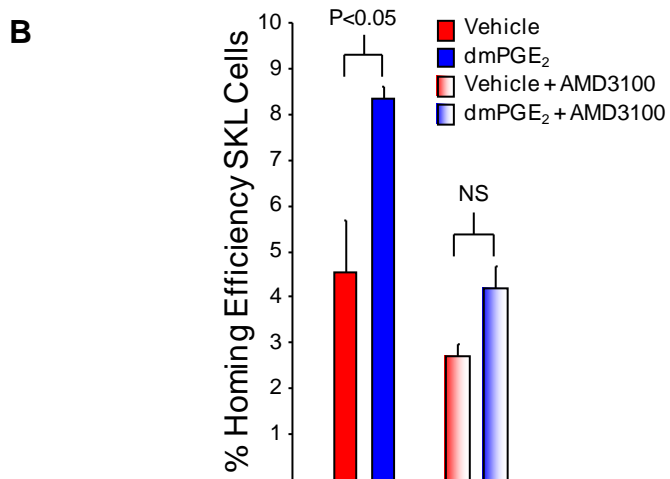
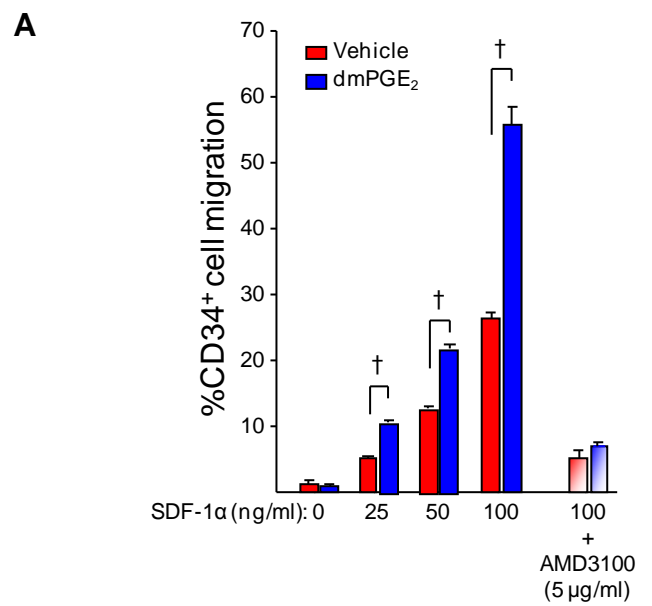


Figure 16. PGE₂ enhancement of migration and homing of HSC and HPC is inhibited by AMD3100.

(A) Freshly isolated UCB CD34⁺ cells were pulsed with dmPGE₂ or vehicle for 2 hours on ice, washed and resuspended in media with 10% HI-FBS and cultured at 37 °C for 16 hours. After incubation, cells were washed, resuspended in RPMI/0.5% BSA and migration to rhSDF-1 α quantitated by flow cytometry. To block the CXCR4 receptor, replicate cells were incubated with 5 μ g/ml AMD3100 for 30 minutes prior to the migration assay. Data are the Mean \pm SEM percentage migration for 3 experiments. [†] P<0.05 for dmPGE₂ treated cells compared to cells treated with vehicle.

(B) Homing efficiency of vehicle and dmPGE₂ treated cells to bone marrow in the absence and presence of 10 μ M AMD3100. Cells were incubated with AMD3100 for 30 minutes prior to the homing assay. Data are expressed as Mean \pm SEM; N=3 mice per group, each assayed individually.

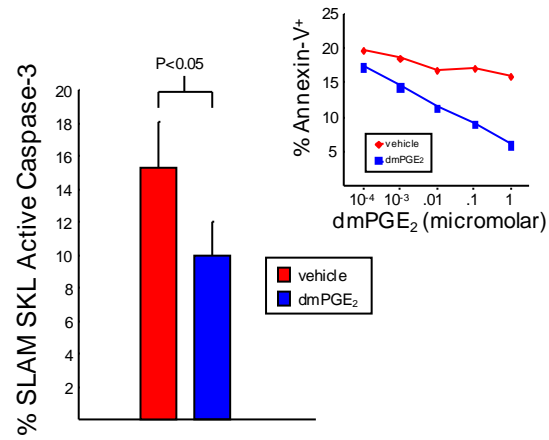
AMD3100 prior to evaluation of *in vivo* homing. PGE₂ pulse-exposure increased homing of SKL cells as described earlier, and incubation of vehicle or dmPGE₂ pulsed cells with AMD3100 significantly reduced SKL cell homing (Figure 16B) and abrogated the improved homing efficiency of dmPGE₂ pulsed cells. Pulse-exposure to dmPGE₂ enhanced SKL cell homing efficiency by 2.6 ± 0.3 fold ($P < 0.05$), which was reduced to 1.3 ± 0.2 fold ($P = \text{NS}$) in the presence of AMD3100. AMD3100 reduced overall homing by $42 \pm 5\%$ (range 31-64), consistent with previous reports (Christopherson et al., 2004; Fukuda et al., 2007).

PGE₂ decreases HSC apoptosis and increases Survivin

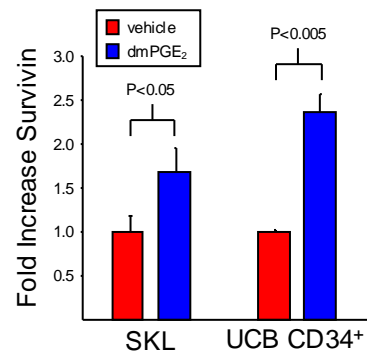
PGE₂ treatment produces a ~4-fold increase in HSC and CRU frequency (Figure 5), but only a ~2-fold enhancement in homing (Figure 11), which suggests that other mechanisms are involved in enhanced engraftment. Apoptosis is an important regulatory process in normal and malignant hematopoiesis (Koury, 1992) and PGE₂ has been implicated in anti-apoptotic signaling (Fernandez-Martinez et al., 2006; George et al., 2007). Moreover, activation of cAMP, a downstream signaling molecule of EP receptors, inhibits apoptosis in CD34⁺ cells (Negrotto et al., 2006). We hypothesized that dmPGE₂ treatment affects survival and/or proliferation of HSC, contributing to enhanced engraftment. Under conditions of reduced serum concentration, dmPGE₂ pulse-exposure significantly reduced intracellular active caspase-3 in SLAM SKL cells (Figure 17A). Dose ranging studies using Annexin-V as an additional marker of

Figure 17

A



B



C

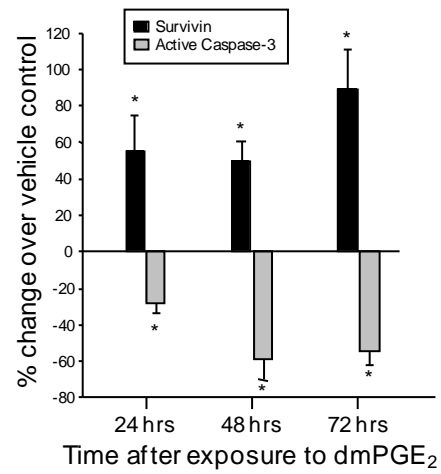


Figure 17. PGE₂ decreases apoptosis, increases Survivin expression and decreases active caspase-3 in HSC.

(A) Lin^{neg} bone marrow cells were treated with dmPGE₂ or vehicle and cultured in media supplemented with 2% HI-FBS without growth factors for 24 hours to induce apoptosis. Cultured cells were stained for SKL and SLAM and PE-anti-active caspase-3 and/or FITC-Annexin-V and the proportion of SKL or SLAM SKL cells undergoing apoptosis determined by FACS. (Inset) dose response analysis of the effects of dmPGE₂ on SKL cell apoptosis.

(B) Fold increase in MFI for intracellular Survivin levels in control and dmPGE₂ pulsed murine SKL and human CD34⁺ cells 24 hours after treatment. Data are Mean ± SEM from 3 experiments, N=3 mice per group, each assayed individually, or are representative of 3 separate UCB samples.

(C) Intracellular Survivin and active caspase-3 levels in SKL cells 24, 48, and 72 hours post-treatment with dmPGE₂. Data expressed as Mean ± SEM for 3 experiments; N=3-6 mice per group, each assayed individually. *P<0.05.

apoptosis indicated that dmPGE₂ inhibited apoptosis in a dose-dependent fashion, reaching ~65% inhibition at 1 μ M (Figure 17A-Inset).

Our laboratory previously showed that the inhibitor of apoptosis protein Survivin regulates apoptosis and proliferation of HSC (Fukuda and Pelus, 2001; Fukuda and Pelus, 2002) and PGE₂ has been reported to alter Survivin levels in cancer cells (Baratelli et al., 2005; Krysan et al., 2004). We therefore evaluated if PGE₂ affected Survivin expression in HSC and HPC. At 24 hours post dmPGE₂ treatment, intracellular Survivin levels were significantly higher in murine SKL cells and human CD34⁺ UCB cells (1.7 and 2.4 fold, respectively) compared to control (Figure 17B). QRT-PCR analysis of treated SKL cells similarly indicated elevated Survivin mRNA compared to control (2.94 fold). In a kinetic analysis, decreased active caspase-3 coincident with an increase in Survivin was seen at 24, 48, and 72 hours post-exposure of SKL cells to dmPGE₂ compared to control (Figure 17C), consistent with the caspase-3 inhibiting activity of Survivin (Tamm et al., 1998).

PGE₂ increases HSC proliferation

Survivin regulates HSC entry into and progression through cell cycle (Fukuda et al., 2002; Fukuda and Pelus, 2001). Furthermore, β -catenin, implicated in HSC proliferation and self-renewal (Fleming et al., 2008), lies downstream of EP receptor pathways (Regan, 2003). The ability of PGE₂ to modulate these cell cycle regulators suggests that an increase in HSC self-

renewal and proliferation might contribute to the enhanced engraftment of dmPGE₂ pulsed cells. To test this hypothesis, we analyzed the cell cycle status of dmPGE₂ or vehicle pulsed SKL cells *in vitro*. Pulse-exposure to dmPGE₂ increased SKL cell cycling (Figure 18A), with 60% more SKL cells in G₁ + S/G₂M phase of the cell cycle after dmPGE₂ treatment compared to controls. To evaluate the effect of dmPGE₂ exposure on primitive, quiescent HSC, we performed additional *in vitro* studies using SLAM SKL cells. Similar to SKL cells, *in vitro* dmPGE₂ pulse-exposure significantly increased the proportion of SLAM SKL cells in cell cycle (G₁ + S/G₂M) by 24% (Table 4). No significant effect on cell cycle rate of KL or Lin^{neg} cells was seen (not shown); suggesting that dmPGE₂ selectively increases HSC cycling state.

To confirm the effect of dmPGE₂ on enhancement of HSC cell cycle observed *in vitro*, bone marrow cells were pulsed with dmPGE₂ and injected into congenic mice treated with BrdU post-transplant, and the proportion of donor BrdU⁺ SKL cells determined 16 hours later (Figure 18B). A ~2-fold increase in the proportion of homed SKL cells in S + G₂/M phase was observed for cells pulsed with dmPGE₂ prior to transplant, confirming that short-term exposure of HSC to dmPGE₂ stimulates HSC to enter and progress through cell cycle *in vivo*.

Figure 18

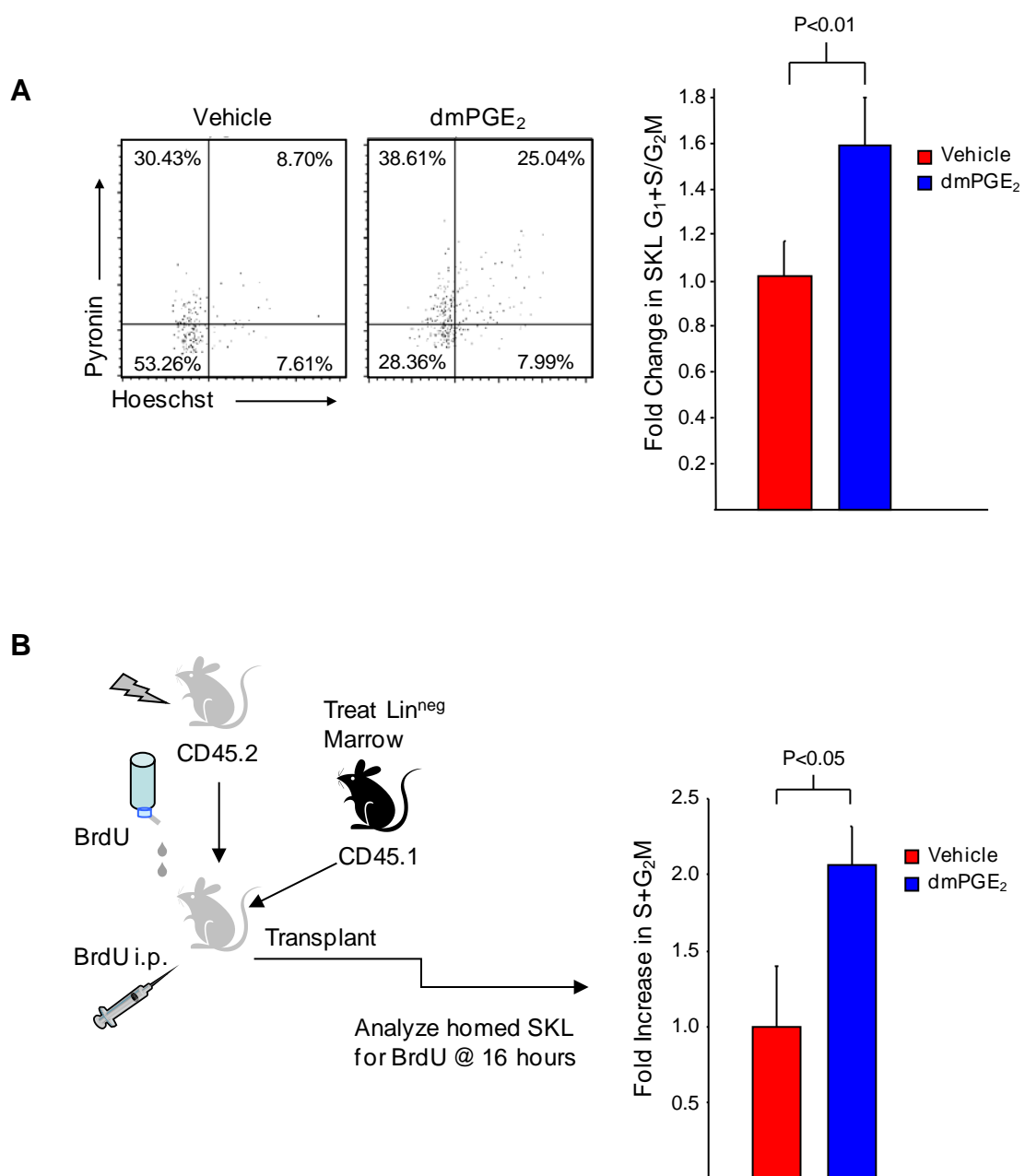


Figure 18. PGE₂ increases the proliferation of SKL cells.

(A) Lin^{neg} cells were treated with either vehicle or 1 μ M dmPGE₂ for 2 hours, washed and cultured in media with rmSCF, rhFlt3 and rhTPO. After 20 hours, cells were stained for SKL and Hoechst-33342 and Pyronin-Y. The proportion of SKL cells in cell cycle was quantitated by FACS. Representative flow plot showing cell cycle distribution of gated SKL cells and combined data for fold increase in cell cycle for dmPGE₂ treated cells compared to vehicle control from 3 experiments, Mean \pm SEM, N=9 mice, each assayed individually.

(B) CD45.1 Lin^{neg} bone marrow cells were treated with dmPGE₂ or vehicle and transplanted into lethally irradiated CD45.2 mice. Immediately after transplantation, BrdU was provided in drinking water and administered by IP injection. Bone marrow was analyzed 16 hours later and the proportion of CD45.1⁺ SKL cells that were BrdU⁺ was analyzed by FACS analysis. Data are Mean \pm SEM, N=5 mice per group, each assayed individually.

Table 4

Effects of short term *in vitro* exposure of SLAM SKL cells to
dmPGE₂ on cell cycle

| SLAM SKL Cells ^a | | | | |
|------------------------------|----------------|----------------|--------------------|-------------------------------|
| <i>In vitro</i> treatment | G ₀ | G ₁ | S+G ₂ M | % cells in cycle ^b |
| Vehicle | 63.4±2.5 | 2.6±0.7 | 33.8±2.1 | 36.4±2.4 |
| 1 µM dmPGE ₂ | 54.8±2.2 * | 6.8±1.9 * | 38.4±1.6 * | 45.2±2.2 * |

Table 4. Effects of short term *in vitro* exposure of SLAM SKL cells to dmPGE₂ on cell cycle.

- a. Lin^{neg} cells treated with either 1 μ M dmPGE₂ or vehicle for 2 hours and cultured in the presence of growth factors (50 ng/ml rmSCF, 100 ng/ml each of rhFlt-3 and rhTPO) for 20 hours, were stained for SLAM SKL, Hoechst-33342 and Pyronin-Y and the proportion of SLAM SKL cells in G₀, G₁, S and G₂/M phase of the cell cycle determined by quantitation of DNA and RNA content by FACS. Data are Mean \pm SEM for N=9 mice, each assayed individually.
- b. Percentage of cells in G₁+S+G₂M; combined data for N=9 mice.
- * P<0.05 compared to vehicle control.

Discussion:

It is well documented that PGE₂ participates in regulation of hematopoiesis, both inhibiting myelopoiesis *in vitro* (Pelus et al., 1979) and *in vivo* (Gentile et al., 1983) and promoting erythroid and multi-lineage colony formation (Lu et al., 1984; Lu et al., 1987) and enhancing proliferation of CFU-S (Feher and Gidali, 1974) and CFU-GM (Verma et al., 1981). In addition, PGE₂ stimulates production of cycling HPC from the quiescent bone marrow compartment (Pelus, 1982), suggesting that PGE₂ has biphasic effects on hematopoiesis. These studies implicated PGE₂ in stem cell function, but did not directly evaluate HSC. Moreover, one cannot rule out that inhibition of colony formation by PGE₂ resulted from modulation of HSC commitment to self-renewal versus differentiation, thus reducing colony formation. Recently, *ex vivo* exposure of bone marrow cells to PGE₂ was shown to facilitate murine hematopoietic cell engraftment (North et al., 2007), validating previous studies that PGE₂ enhances HPC production and extending the role of PGE₂ to stimulation of HSC function. However, the mechanism by which PGE₂ produced this effect was not defined. We now demonstrate, for the first time, that PGE₂ has direct and stable effects on long-term repopulating HSC, as determined by serial transplantation, and facilitates HSC engraftment by increasing CXCR4, enhancing migration to SDF-1 α and homing to bone marrow, up-regulating Survivin expression that blocks HSC apoptosis, and increasing the proportion of LTR-HSC entering into and progressing through cell cycle.

Direct comparison in competitive transplant models showed that short-term exposure of HSC to PGE₂ produces a ~4-fold competitive advantage, consistent with published results (North et al., 2007). However, previous studies showed a maximal effect on HSC frequency at 12 weeks post-transplant with reduced HSC frequency at 24 weeks, suggesting a greater effect on short-term rather than long-term repopulating cells. Our studies show that PGE₂-induced enhancement of HSC frequency was stable throughout a >20 week period and was maintained in secondary transplants through 24 weeks, clearly indicating a sustained effect on LTRC. The reasons for this difference in repopulating stability are not clear, but may relate to more precise head-to-head quantitation of HSC competition in our model.

Full hematopoietic reconstitution was observed in serially transplanted recipients using either control or PGE₂ treated cells, indicating no adverse impact of PGE₂ on HSC self-renewal. Maintenance of the PGE₂ treated graft through four separate serial transplantations clearly indicates that LT-HSC were enhanced, and not the recently described IT-HSC (Benveniste et al., 2010). In fact, a trend towards increased LTRC activity was seen, indicating that the enhancing effect of short-term PGE₂ exposure on HSC observed in primary transplants was long lasting, since no additional treatment was performed on cells or animals before secondary transplants. However, our long-term competitiveness assay, in which we equalized HSC content from previously treated PGE₂ and vehicle grafts, demonstrates that there is not an inherent

competitive advantage to the PGE₂ treated grafts, but rather enhancements in the serial transplants were simply mediated by increased numbers of HSC in the primary graft, presumably by increased homing and self-renewal. While it is commonly assumed that a single HSC compartment gives rise to all hematopoietic lineages, recent studies have demonstrated the presence of normal HSC biased towards lymphoid or myeloid differentiation (Muller-Sieburg and Sieburg, 2006a). In secondary transplants, we observed a myeloid bias in mice transplanted with PGE₂ treated HSC, suggesting a possible selective effect of PGE₂ on myeloid-biased HSC. However, white blood cell counts in serial transplanted mice have remained within normal ranges, and this myeloid shift was not observed in subsequent transplants.

While it was suggested that PGE₂ does not affect HSC homing, earlier studies evaluated WBM (North et al., 2007) and did not specifically assess HSC or HPC. When evaluating total transplanted cells we also observed no difference in homing efficiency between control and PGE₂ treated cells; however, enhanced homing of SKL cells by PGE₂ was clearly evident. Furthermore, enhanced homing efficiency of PGE₂ treated, sorted SKL cells was observed, suggesting a direct effect on HSC. PGE₂ also enhanced homing of human CD34⁺ UCB in immunodeficient NSG mice, strongly indicating translation of HSC enhancement to human stem cell grafts. Although more primitive populations of HSC than defined by SKL can be identified, (e.g. CD34⁻ SKL and SLAM SKL), the small number of homed events that can ultimately be detected using these markers

precludes the ability to define effects of PGE₂ on these extremely rare cells *in vivo* in individual mice as we performed. The fact that we see similar activities of PGE₂ on LTRC and on SKL and SLAM SKL cells in a number of assays of HSC function without significant effects on the progenitor cell-enriched KL cell population, indicates that the SKL cell fraction is a valid indicator of the effects of PGE₂ on HSC homing.

PGE₂ treatment increased SKL CXCR4 mRNA and surface expression, consistent with effects of PGE₂ on CXCR4 in CD34⁺ cells (Goichberg et al., 2006). This increase in CXCR4 corresponds directly with a functional increase in chemotaxis to SDF-1 α , and chemotaxis was blocked using AMD3100. In addition, AMD3100 significantly reduced the enhancing effect of PGE₂ on homing *in vivo*; suggesting that increased CXCR4 expression and chemo-attraction to marrow SDF-1 α are largely responsible for the enhanced homing effect, although additional effects on adhesion molecule expression or function cannot be excluded. We also found elevated mRNA and protein levels of Survivin, with concomitant reduced active caspase-3 in PGE₂ treated SLAM SKL cells. It is likely that enhanced HSC survival, mediated through Survivin, also contributes to enhanced engraftment.

Pulse-exposure to PGE₂ increased the proportion of HSC in cell cycle by ~2-fold, with increased frequency of HSC, CRU and homed BrdU⁺ SKL cells and maintenance of enhanced HSC frequency in serial transplants, suggesting that PGE₂ pulse-exposure initiated at least a single round of HSC self-renewal. EP2

and EP4 receptor activation can result in phosphorylation GSK-3 and increased β -catenin signaling (Regan, 2003), which is downstream of the Wnt pathway that has been implicated in HSC survival and self-renewal (Fleming et al., 2008; Khan and Bendall, 2006). Signaling by PGE₂ through EP4 can directly increase β -catenin and synergistic cross-talk between COX2 and Wnt pathways has been suggested (Wang et al., 2004). Further exploration of specific signaling pathways and EP receptors involved in mediating the effects of PGE₂ may refine our understanding of the role of PGE₂ on HSC function. While it has been suggested that cycling cells have reduced marrow homing, which may be the result of triggered apoptosis (Jetmore et al., 2002), it is clear that PGE₂ treated cells have both enhanced homing and enhanced migration, despite their enhanced cycling. This may be explained by the increase in CXCR4 migratory response overcoming deficits in cycling-cell homing and/or increased homing occurring before an increase in cycling. Additionally, PGE₂ may protect homed cycling HSC from apoptosis, thus allowing for simultaneous enhanced homing, survival and proliferation in these cells.

Our laboratory previously reported that Survivin is required for HSC to enter and progress through cell cycle (Fukuda et al., 2002; Fukuda and Pelus, 2002) and deletion in conditional knockout mice indicates Survivin is required for HSC maintenance (Leung et al., 2007). Survivin also facilitates HSC cell cycle through p21^{WAF1/CDKN1} (Fukuda et al., 2004), known to be involved in HSC function (Cheng et al., 2000), and blocks caspase-3 activity (Tamm et al., 1998),

recently implicated in HSC self-renewal (Janzen et al., 2008). Our findings that PGE₂ up-regulates Survivin, which is consistent with previous reports in cancer (Krysan et al., 2004) and dendritic cells (Baratelli et al., 2005), and decreases intracellular levels of active caspase-3 in primitive HSC, suggest that the Survivin pathway may also be involved in the effects of PGE₂ on self-renewal. It is interesting to note that Survivin (Peng et al., 2006) and CXCR4 (Staller et al., 2003) transcription are both up-regulated by the transcription factor hypoxia-inducible factor-1 α (HIF-1 α), which can be stabilized by PGE₂ (Piccoli et al., 2007), potentially linking these PGE₂ responsive pathways.

In summary, we have defined specific mechanisms of action and new pathways for enhancement and regulation of HSC function by PGE₂. The 4-fold increase in HSC frequency and engraftment produced by exposure to PGE₂ results from the cumulative effect of a 2-fold increase in HSC homing and a 2-fold increase in HSC cell cycle activity. Although the precise signaling pathways remain to be determined, enhanced engraftment involves up-regulation of CXCR4 and Survivin, with subsequent increased chemotactic response to SDF-1 α and reduced apoptosis. The ability to easily improve homing and survival/proliferation of HSC by short-term PGE₂ exposure is exciting from a clinical perspective, especially in transplant settings where insufficient or low HSC numbers are found, e.g. UCB and some mobilized peripheral blood stem cell (PBSC) products. Our limiting dilution transplant studies show that equivalent engraftment is achieved with one-fourth the number of PGE₂ treated

cells compared to controls, supporting a use for PGE₂ when HSC numbers are limiting. Homing and migration studies utilizing UCB CD34⁺ cells clearly suggest potential translation to human hematopoietic grafts. Lastly, it will be interesting to determine if enhanced engraftment/recovery can be achieved by administering PGE₂ *in vivo* or if PGE₂ used *in vivo* can further facilitate engraftment of HSC exposed to PGE₂ *ex vivo*. In COX2 knockout mice, recovery from 5-fluorouracil (5-FU) is delayed (Lorenz et al., 1999) suggesting that COX2 activation and subsequent PGE₂ production may be critical for HSC expansion. Analysis of the effects of COX inhibition is explored in Chapter 3, and new findings on COX inhibition in irradiated animals are discussed in Chapter 4.

Chapter 3. Modulation of Eicosanoid Signaling to Enhance Hematopoietic Expansion and Mobilization

Introduction:

As discussed in Chapter 1, mobilized adult HSC and HPC are widely used for autologous and allogeneic transplantation and have improved patient outcomes. G-CSF-mobilized PBSC are associated with more rapid engraftment, shorter hospital stay (Jansen et al., 1999; Kennedy et al., 1993; McQuaker et al., 1997; Nemunaitis et al., 1995), and in some circumstances, superior overall survival compared to bone marrow (Stem Cell Trialists' Group, 2005).

Administration of G-CSF for 5-7 days to patients and normal donors is associated with morbidity in the form of bone pain, nausea, headache and fatigue (Anderlini et al., 1998; Anderlini et al., 2001; Fortanier et al., 2002; Rowley et al., 2001), which can be lifestyle disruptive in normal volunteers. G-CSF is associated with serious toxicity, including enlargement of the spleen (Platzbecker et al., 2001; Stroncek et al., 2003) and splenic rupture (Balaguer et al., 2004; Becker et al., 1997; Falzetti et al., 1999; Kroger et al., 2002) in normal donors. The pro-coagulant effects of G-CSF increase risk of myocardial infarction and cerebral ischemia in high-risk individuals (Hill et al., 2005; Lindemann and Rumberger, 1993). G-CSF is contraindicated in patients with Sickle Cell Disease for its potential to precipitate sickle crisis (Adler et al., 2001; Kang et al., 2002) precluding its use as a mobilizing agent in this patient population, which has a

potential negative impact on utility of adult HSC gene therapy for these patients. Poor mobilization in response to G-CSF occurs in 25% of patients, particularly those with lymphoma and multiple myeloma (Stiff et al., 2000) and 15% of normal donors (Anderlini et al., 1997), requiring extended aphereses (Schmitz et al., 1995). The incidence of chronic GVHD is higher (Couban et al., 2002; Cutler et al., 2001; Mohty et al., 2002) for G-CSF-mobilized PBSC than bone marrow. Hence, there continues to be a search for safe and effective mobilizing agents to expand the use of hematopoietic grafts and PBSC transplantation.

Expanded application of allogeneic transplantation for malignant disease, tolerance induction for solid organ transplant, and HSC based gene therapies that do not provide a competitive advantage to the graft, require larger doses of PBSC to ensure durable engraftment, acceptable leukocyte recovery kinetics and low incidence of GVHD (Aversa et al., 1998; Reisner and Martelli, 2000). Optimum CD34⁺ cell dose for allogeneic transplantation remains unknown. Studies suggest that doses $>3 \times 10^6/\text{kg}$ are associated with reduced morbidity and mortality (Bittencourt et al., 2002), however higher CD34⁺ cell doses result in more rapid engraftment, less morbidity and better survival rates (Pulsipher et al., 2009), particularly for patients with disease at high risk of relapse (Nakamura et al., 2008; Panse et al., 2005). The small molecule CXCR4 antagonist AMD3100 has been used successfully to mobilize PBSC from normal donors for allogeneic transplant (Devine et al., 2008). However, the use of AMD3100 alone for

mobilization of HSC and HPC is unlikely to yield sufficient CD34⁺ cells for over half of patients undergoing allogeneic transplant.

As discussed in Chapter 2, we have identified new roles for PGE₂ in regulating HSC homing to bone marrow, survival, and stem cell self-renewal. These findings, coupled with earlier findings on the inhibition of HPC expansion by PGE₂ (Gentile and Pelus, 1988; Kurland et al., 1978; Kurland et al., 1979; Pelus et al., 1979; Pelus et al., 1981; Pelus et al., 1983; Pelus et al., 1988; Pelus and Gentile, 1988) led to our hypothesis that inhibition of PGE₂ synthesis with NSAIDs would block the inhibition of HPC expansion, and would reduce HSC and HPC tethering within the bone marrow to facilitate mobilization. In this Chapter, we provide evidence demonstrating that NSAIDs alone and in combination with mobilizing agents facilitate mobilization of HSC and HPC. NSAID facilitated grafts have superior engraftment capability in lethally irradiated mice, with long-term repopulation. We also show enhancement of HSC and HPC mobilization in NSAID treated baboons. These positive effects on HSC and HPC regulation require inhibition of both COX1 and COX2 enzymes, and we show here that HPC expansion and mobilization is elicited through a reduction in PGE₂ EP4 receptor signaling. Our results define a novel role for NSAIDs and suggest that the addition of NSAIDs, particularly Meloxicam, to current mobilization regimens will increase HSC and HPC yield. In addition, we have identified novel mechanisms mediated by EP receptors and show that HPC expansion and hematopoietic mobilization can be enhanced *in vivo* through antagonism of the

EP4 receptor, defining a new pharmaceutical target for hematopoietic mobilization and transplantation.

Materials and Methods:

Mice and baboons

C57Bl/6 (CD45.2) mice were purchased from Jackson Laboratories (Bar Harbor, ME). B6.SJL-PtrcAPep3B/BoyJ (BOYJ) (CD45.1) mice were bred in-house. Mice used in transplant studies received Doxycycline feed for 30 days post-transplant. The Animal Care and Use Committee of IUSM approved all protocols. Female olive baboons, *Papio anubis*, within the weight range of 16-19 kg, were housed individually in conventional caging and holding rooms of the Biological Resources Laboratory, a centralized animal facility for the University of Illinois at Chicago Medical Center, Chicago, IL, which is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care-International. Baboons were maintained in rooms conditioned to 18.5-24.0 °C with an average relative humidity of 30% and provided a 12-hour light, 12-hour dark full-spectrum light cycle. Baboons were fed with commercial 15% protein monkey chow (Teklad 8714, Harlan, Indianapolis, IN) supplemented with fresh fruit and foraging mix and had *ad libitum* access to tap water. The conducted research was approved by the University of Illinois at Chicago Animal Care and Use Committee. The use of baboon PBSC was similarly approved by the Indiana University IACUC and IRB.

Peripheral blood and bone marrow acquisition and processing

Peripheral blood from mice was obtained by cardiac puncture following CO₂ asphyxiation using an ethylenediaminetetraacetic acid (EDTA) rinsed syringe. Blood was transferred to tubes containing EDTA for complete blood cell (CBC) analysis. CBC analysis was performed on a Hemavet 950FS (Drew Scientific). Peripheral blood mononuclear cells (PBMC) were prepared by centrifugation over Lympholyte Mammal (Cedarlane Laboratories Ltd, Hunby, Ontario, Canada) at 800g for 30-40 minutes at room temperature, followed by triplicate washes. Bone marrow cells were harvested by flushing femurs with ice-cold PBS and single-cell suspensions prepared by passage through a 26-gauge needle. For baboons, peripheral blood was obtained from the femoral vein of baboons anesthetized with an intramuscular injection of 10 mg/kg ketamine hydrochloride (Bionichepharma, Lakeforest, IL). Blood was collected into 10 ml sterile EDTA vacutainers (Becton, Dickinson and company, Franklin, NJ) and transported on ice to the IUSM campus for analysis. Complete blood counts with differential counts were performed on a Hemavet 950FS. Peripheral blood was then diluted 1:3 with PBS and mononuclear cells were isolated using Ficoll-Paque™ Plus (Amersham Biosciences), per manufacturer's protocol.

Colony assays

Bone marrow cells or PBMC were resuspended in McCoy's 5A modified media supplemented with 100 U/ml penicillin, 100 µg/ml streptomycin, 0.6 X modified essential medium (MEM) vitamin solution, 1 mM sodium pyruvate, 0.8 X MEM essential amino acids, 0.6 X MEM nonessential amino acids, 0.05% sodium bicarbonate (all from Gibco), serine, asparagine, glutamine mixture and 15% HI-FBS (Hyclone Sterile Systems, Logan, UT) as described (King et al., 2001; Pelus et al., 1979). Cells were mixed with 0.3% agar (Difco Laboratories, Detroit, MI) in McCoy's 5A medium with 10 ng/ml rhGM-CSF and 50 ng/ml rmSCF (R&D). PBMC were cultured at 2×10^5 cells per ml and bone marrow cells at 5×10^4 cells per ml. All cultures were established in triplicate from individual animals, incubated at 37 °C, 5% CO₂, 5% O₂ in air for 7 days and colonies quantitated by microscopy. In some experiments, total CFC including CFU-GM, BFU-E and CFU-GEMM were enumerated in 1% methylcellulose/IMDM containing 30% fetal bovine serum, 1 U/ml recombinant human erythropoietin (EPO), 10 ng/ml rhGM-CSF and 50 ng/ml rmSCF as described (Broxmeyer et al., 2007a; Fukuda et al., 2007). For baboon colonies, similar assays were performed using recombinant human growth factors.

Flow cytometry

All antibodies were purchased from BD Biosciences unless otherwise noted. For detection of SKL cells, we used streptavidin conjugated with PE-Cy7

(to stain for biotinylated MACS[®] lineage antibodies (Miltenyi), c-kit-APC, Sca-1-PE or APC-Cy7, CD45.1-PE, CD45.2-FITC. For SLAM SKL, we utilized Sca-1-PE-Cy7, c-kit-FITC, CD150-APC (eBiosciences, San Diego, CA), CD48-biotin (eBiosciences) and streptavidin-PE. CXCR4 expression was analyzed using biotinylated Lineage antibodies, streptavidin-PECy7, c-kit-APC, Sca-1-APC-Cy7, and CXCR4-PE. For baboon CD34 analysis, CD34-PE (Clone 563) was used. Analyses were performed on an LSRII flow cytometer (BD).

Peripheral blood mobilization

Several different mobilization strategies were employed throughout this dissertation. The specific details of dosing and schematics of dosing regimens are shown on the data figures or included in the figure legends. In general, mice were given subcutaneous treatments of vehicle, NSAID (at varying doses), G-CSF (50µg/kg, twice a day for 4 days), or G-CSF plus NSAID. For studies exploring mobilizing agents other than G-CSF, mice were treated with AMD3100 (5 mg/kg day 5; single injection), or AMD3100 plus GROβ (5 mg/kg and 20 mg/kg respectively, day 5; single co-injection), and peripheral blood harvested at 1 hour, or 15 minutes after injection, respectively. For comparisons of multiple different NSAIDs, all NSAIDs were dosed by oral gavage. Animals were restrained by tightly scruffing with the non-gavage hand, and oral gavage was performed using a 1.5 inch, curved, 20-gauge, stainless steel feeding needle with a 2.25 mm ball (Braintree Scientific, Braintree, MA). Each gavage treatment was given in a 0.2 ml bolus (10 ml/kg) of 0.5% methyl cellulose (Methyl Cellulose M-0512, Sigma-

Aldrich, St. Louis, MO) with an NSAID suspended in solution. For EP receptor analysis, mice were mobilized with G-CSF in combination with Meloxicam, AH6809 (EP1-3 antagonist, 10 µg per mouse, *ip*, 4 days), AH23848 (EP4 antagonist, 10 µg per mouse, *ip*, 4 days), L-161,982 (EP4 antagonist, 10 µg per mouse, *ip*, 4 days) or G-CSF plus Meloxicam and an EP2, EP1/3 or EP4 agonist (10 µg per mouse, *ip*, 4 days) or dmPGE₂ (10 µg per mouse, *ip*, 4 days). For baboon studies, a baseline bleed was performed for CBC, CD34 and CFC analysis. Two days later, 2 baboons were treated with 10µg/kg G-CSF, and 2 baboons were treated subcutaneously with 10µg/kg G-CSF and 0.2 mg/kg Meloxicam on day 1, followed by 0.1 mg/kg Meloxicam subsequent days, for 5 total days. Blood was collected following treatment regimen for CBC, CD34, and CFC analysis. Following a 2 week resting period, the above procedure was repeated, switching treatment groups for individual baboons. Additionally, after another 2 week resting period, blood was collected before and after a 5 day treatment regimen with Meloxicam and CBC, CD34, and CFC were analyzed.

Limiting dilution competitive transplantation

CD45.1 mice were mobilized with a standard 4 day regimen of G-CSF, or G-CSF plus a 4 day regimen of Meloxicam (6 mg/kg). In some studies designed to evaluate timing and duration of NSAID dosing in combination with G-CSF, initiation of the NSAID regimen preceded G-CSF and was staggered such that NSAID administration ended simultaneous with the G-CSF regimen (no stagger), 1 day prior to G-CSF (1 day stagger) or 2 days prior to G-CSF (2 day stagger)

(regimens as depicted in the corresponding data figure). On day 5, PBMC were acquired and transplanted at 1:1, 2:1, 3:1 or 4:1 ratios with 5×10^5 C57Bl/6J WBM competitors into lethally irradiated C57Bl/6J recipient mice. Peripheral blood chimerism was monitored monthly, and CRU and LT-HSC frequency calculated as described earlier.

Recovery assay

Mice were mobilized with G-CSF or G-CSF plus Meloxicam with staggered dosing as described above and 2×10^6 mobilized PBMC transplanted into cohorts of 10 lethally irradiated recipients per group. A cohort of non-irradiated mice was bled on the same schedule as the experimental treated groups of mice. Every other day, 5 mice from each group were bled (~50µl from a tail snip) and neutrophils and platelets in blood were enumerated using a Hemavet 950FS. Alternate groups of 5 mice were bled on each successive bleeding time point so that mice were only bled once every 4 days. Recovery of neutrophils and platelets to 50% and 100% were determined by comparison to the average neutrophil and platelet counts in the control group throughout the experimental period. After 90 days, mice were sacrificed, bone marrow harvested, and transplanted at a 2.5:1 ratio with 2×10^5 congenic competitors into lethally irradiated recipients to determine long-term repopulating ability of the primary mobilized graft.

Results:

NSAID treatment results in increased HPC

Previous work (Lu et al., 1986; Lu et al., 1987; Pelus, 1982; Pelus, 1984; Pelus, 1989b) and recent studies by us (Hoggatt et al., 2009) and others (Feher and Gidali, 1974; North et al., 2007; Verma et al., 1981) demonstrate a positive role of PGE₂ on HSC function. In addition, our studies extend the effects of PGE₂ to include enhancement of HSC homing, survival and self-renewal (Hoggatt et al., 2009). These results suggest that PGE₂ signaling facilitates HSC maintenance/self-renewal. However, continued exposure to PGE₂ *in vitro* and *in vivo* can inhibit HPC proliferation and differentiation (Gentile and Pelus, 1988; Kurland et al., 1978; Kurland et al., 1979; Pelus et al., 1979; Pelus et al., 1981; Pelus et al., 1983; Pelus et al., 1988; Pelus and Gentile, 1988) and reduce hematopoietic expansion. Previous studies showed that blockade of prostaglandin biosynthesis enhanced HPC expansion induced by IL-1 (Pelus, 1989a). We confirmed and extended these findings and showed that daily administration of the prototypical dual COX inhibitor Indomethacin produced incremental expansion of CFU-GM in the marrow of treated mice reaching ~2-fold after 4 days (not shown). Bone marrow HPC expansion was not observed with COX1 (SC560) or COX2 (Valdecoxib) selective NSAIDs (Figure 19), suggesting that HPC expansion requires inhibition of both COX enzymes. Meloxicam was as effective as Indomethacin in increasing HPC within the bone marrow of treated mice.

Figure 19

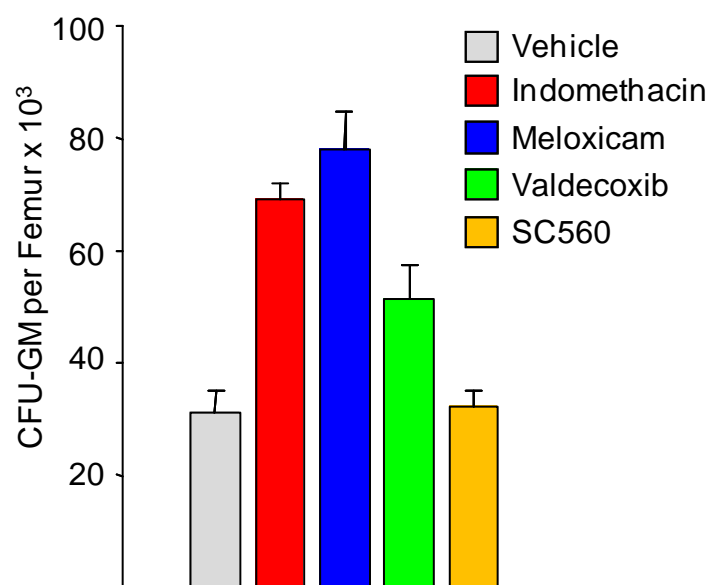


Figure 19. Dual COX inhibitory NSAIDs expand HPC within bone marrow.

Mice were administered dual (Indomethacin, Meloxicam), COX2 selective (Valdecoxib) or COX1 selective (SC560) inhibitors twice daily for 4 days. On day 5, mice were sacrificed, bone marrow from one femur isolated, total nucleated cells counted on a Hemavet 950FS and CFU-GM determined. Data are Mean \pm SEM for N=5 mice per group, each assayed individually.

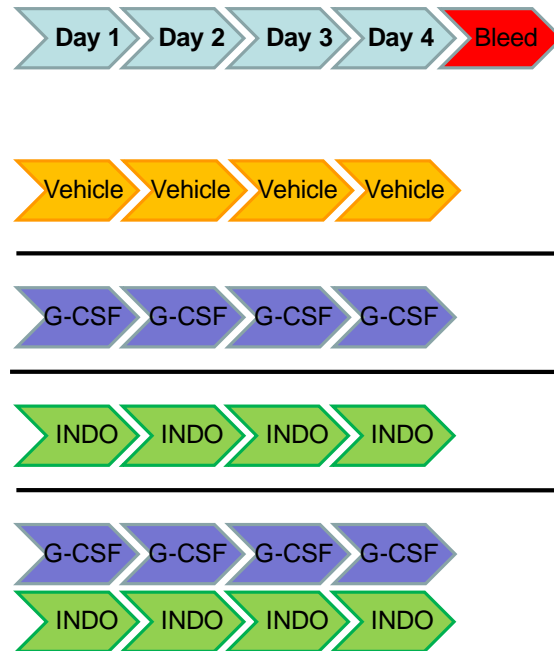
NSAID treatment enhances mobilization of HSC and HPC

In Chapter 2, we presented findings that PGE₂ signaling increased CXCR4 receptor expression (Figure 14) and homing of murine and human HSC to the bone marrow (Figures 11-13). The fact that PGE₂ is produced in bone marrow suggests that it may be involved in maintenance of HSC and HPC CXCR4 expression. Therefore, we reasoned that inhibition of PGE₂ biosynthesis by NSAID administration that reduces PGE₂ signaling that maintains CXCR4 expression and chemoattraction/tethering in the bone marrow, in combination with NSAID expansion of HPC could enhance mobilization of HSC and HPC to peripheral blood. To test this hypothesis, mice were treated with vehicle, Indomethacin, G-CSF, or G-CSF plus Indomethacin twice daily for 4 days (Figure 20A). On day 5, peripheral blood was acquired and analyzed for CFU-GM. Administration of NSAID alone resulted in a marginal, but significant increase in CFU-GM in peripheral blood (Figure 20B, left) and when co-administered with G-CSF synergistically increased mobilization (Figure 20B, right). The LOX inhibitor Baicalein was also evaluated alone and in combination with G-CSF, and had no significant effect on mobilization, indicating that enhancements by NSAIDs are specific to the COX pathway and not due to general eicosanoid inhibition. Multiple experiments demonstrated a ~2-fold increase in CFU-GM mobilization over G-CSF (Figure 21A). Mobilization of BFU-E and CFU-GEMM were similarly enhanced (not shown) indicating that the effect of NSAIDs is not selective for CFU-GM. Immunophenotypic analysis of peripheral blood by flow cytometry

demonstrated significant enhancement in both the SKL and SLAM SKL populations with co-administration of NSAID (Figure 21B), suggesting that both HPC and HSC mobilization are enhanced. Additionally, analysis of CFU-GM colonies from G-CSF mobilized mice compared to G-CSF plus Indomethacin mobilized mice showed a marked shift in monocytic and multi-centric colony formation (Figure 22), which possibly indicates preferential expansion of an altered HPC pool and/or egress of a subset of HPC.

Figure 20

A



B

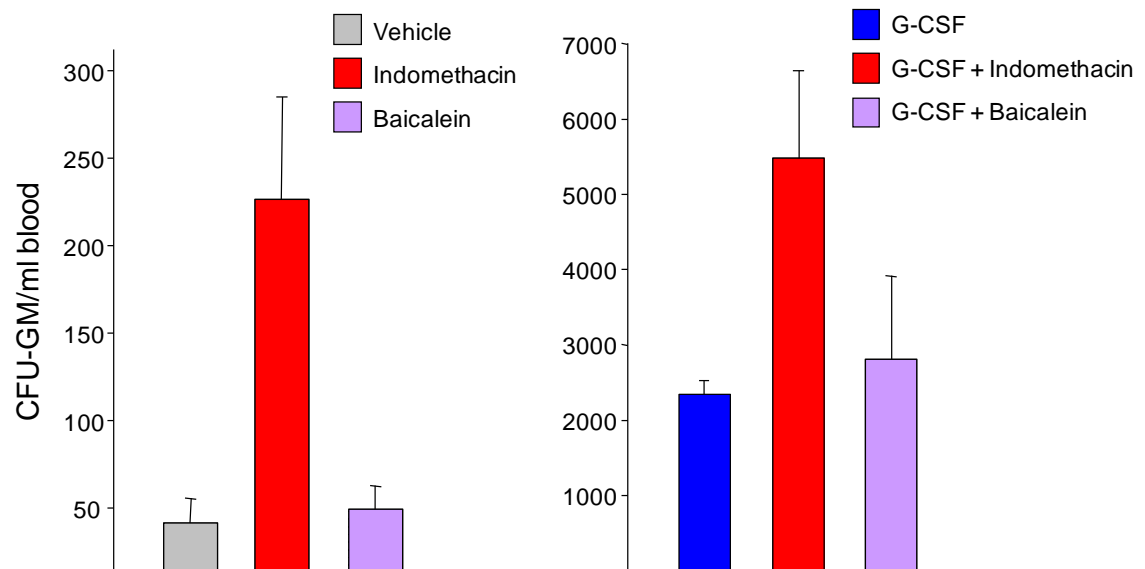


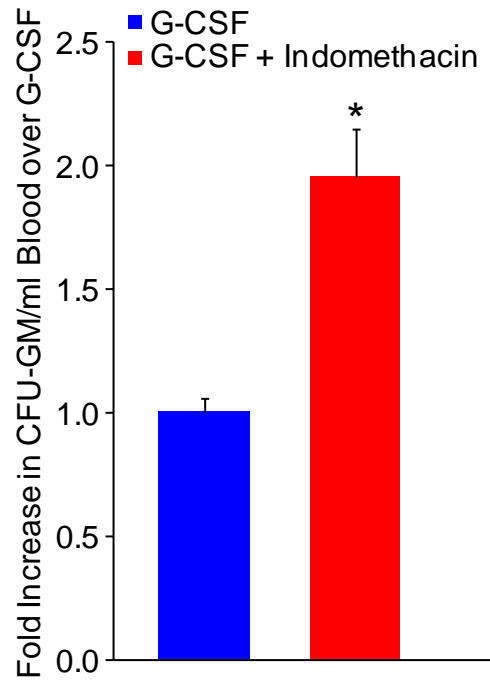
Figure 20. Indomethacin enhances mobilization of CFU-GM.

(A) Schematic of the dosing regimen used to evaluate the mobilization of CFU-GM using G-CSF, Indomethacin, or the combination of G-CSF plus Indomethacin.

(B) Effects of daily subcutaneous administration of 150 µg/kg Indomethacin or 150 µg/kg Baicalein (LOX inhibitor) alone (left panel) or with G-CSF (right panel) for 4 days on CFU-GM mobilization. Data are expressed as Mean ± SEM, CFU-GM mobilized per ml of blood for N=3 mice, each assayed individually.

Figure 21

A



B

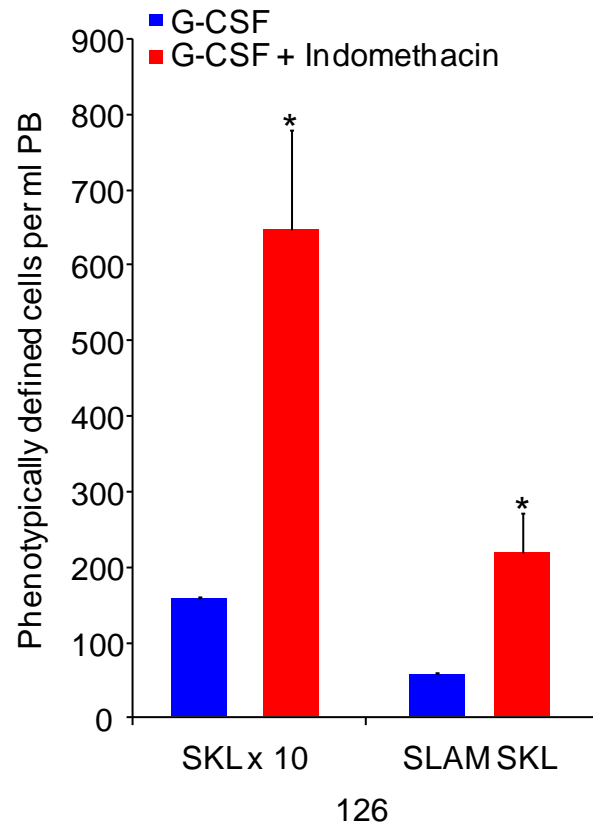


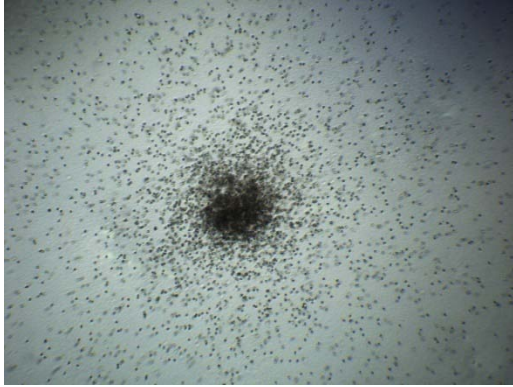
Figure 21. NSAIDs increase mobilization of functionally and phenotypically defined HSC and HPC.

(A) Mobilization of CFU-GM with G-CSF, or the combination of G-CSF and Indomethacin. Data are expressed as Mean \pm SEM from 3 experiments, N=12 mice total per group, each assayed individually. * $P < 0.01$.

(B) Flow cytometric analysis of phenotypically defined HSC in peripheral blood of mice treated with G-CSF or the combination of G-CSF and Indomethacin. Data are expressed as Mean \pm SEM, N=5 mice per group, each assayed individually. * $P < 0.01$.

Figure 22

A



B

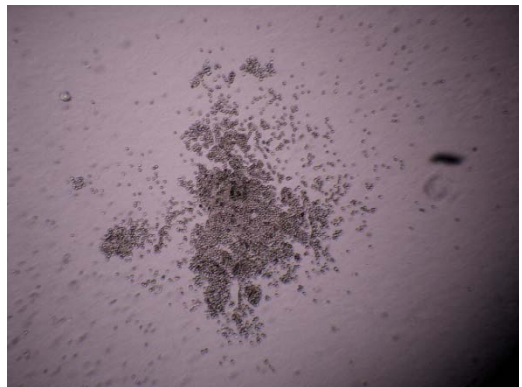
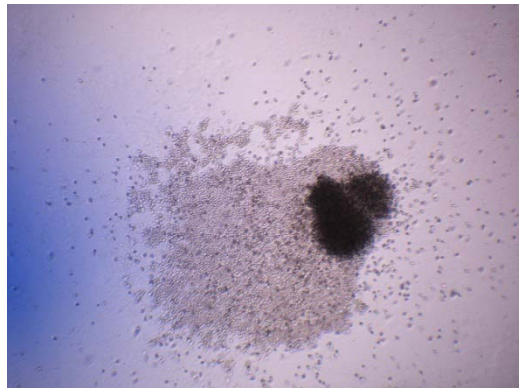
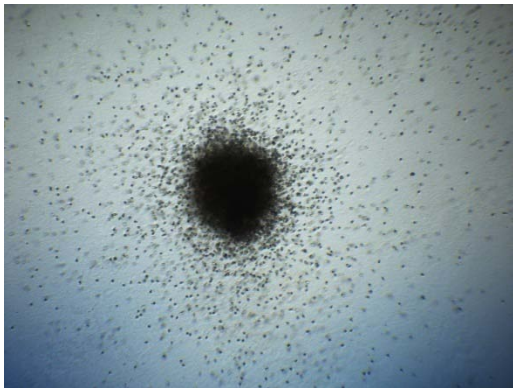
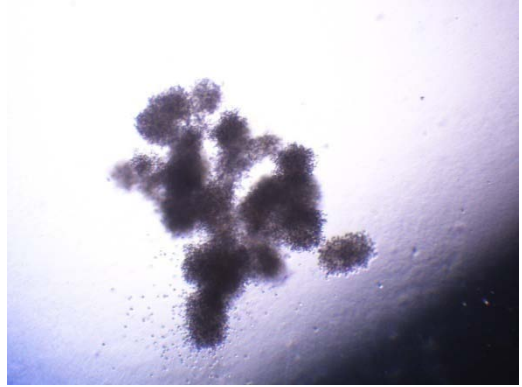


Figure 22. Representative CFU-GM colonies from G-CSF and G-CSF plus NSAID mobilized mice.

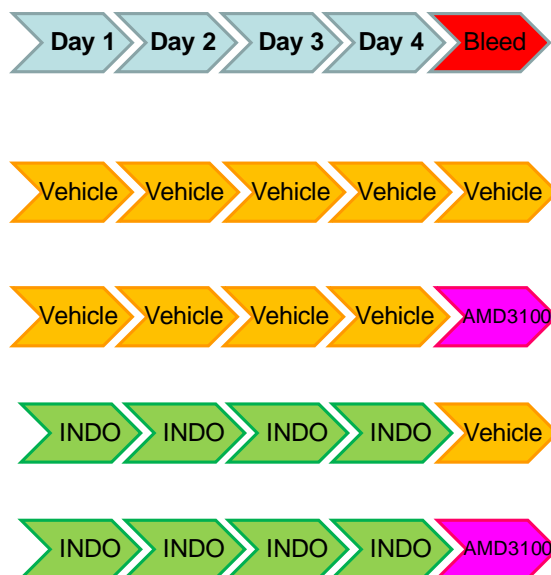
(A) Mice were mobilized with a 4 day regimen of G-CSF, and peripheral blood LDMC plated for CFC in the presence of SCF and GM-CSF. Shown (left side) are “prototypical” colonies found in blood. This representation is not inclusive of all colony types present, but is indicative of the morphology of the predominant colony types present.

(B) Mice were mobilized with a 4 day regimen of G-CSF plus NSAID, and peripheral blood LDMC plated for CFC in the presence of SCF and GM-CSF. Shown (right side) are representations of various “non-prototypical” colonies found as a result of NSAID treatment. This representation is not inclusive of all colony types present, rather it highlights the increase in distinct morphological colony types not routinely observed in control cultures in panel A.

The CXCR4 receptor selective antagonist AMD3100 mobilizes HSC and HPC (Broxmeyer et al., 2005b; Broxmeyer et al., 2007b; Devine et al., 2008; Liles et al., 2003; Liles et al., 2005; Pelus et al., 2005), and has recently received FDA approval for use in combination with G-CSF for use in patients who do not respond well to G-CSF. To evaluate the potential of NSAIDs to enhance mobilization by AMD3100, mice were administered vehicle, Indomethacin, AMD3100, or AMD3100 plus Indomethacin as shown (Figure 23A). As shown previously, Indomethacin increased CFU-GM in peripheral blood (Figure 23B, left), and when co-administered with AMD3100 synergistically enhanced mobilization (Figure 23B, right). To further evaluate the ability of NSAIDs to facilitate mobilization of hematopoietic grafts, mice were treated with vehicle, Indomethacin, AMD3100, G-CSF, AMD3100 plus GRO β (Pelus et al., 2006a; Pelus et al., 2006b; Pelus and Singh, 2008), AMD3100 plus Indomethacin, or G-CSF plus Indomethacin. NSAID co-administration facilitated mobilization with both AMD3100 and G-CSF, with the AMD3100 plus Indomethacin regimen mobilizing CFU-GM with at least the equivalency of G-CSF alone, or AMD3100 plus GRO β (Figure 24). These results demonstrate that facilitation of mobilization with NSAID administration is independent of the mobilization mechanisms of G-CSF, suggesting that bone marrow expansion with an NSAID can be used concurrently with any mobilizing agent to increase hematopoietic yield.

Figure 23

A



B

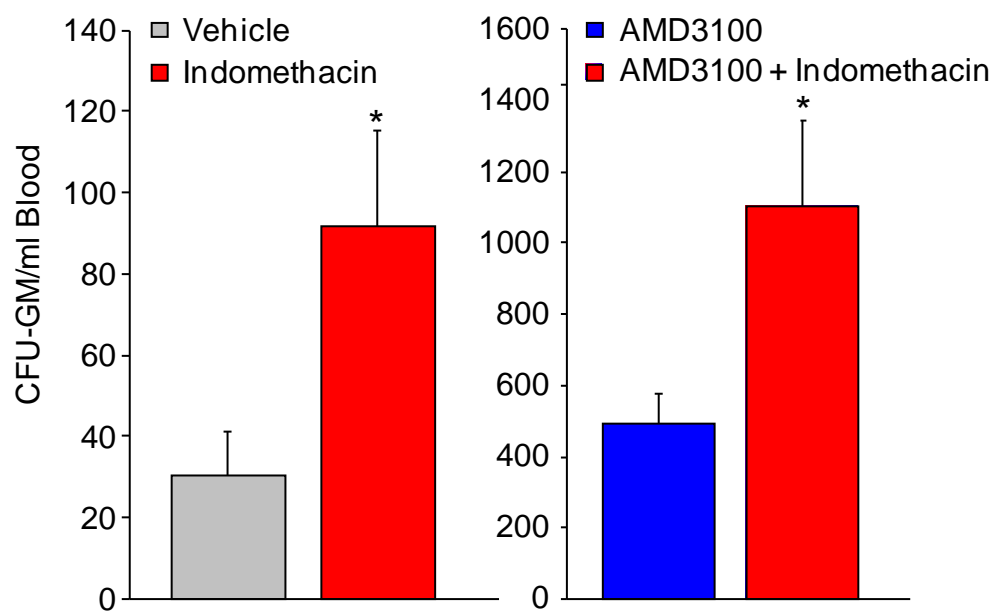


Figure 23. NSAIDs synergistically increase mobilization with AMD3100.

(A) Schematic of dosing regimen used to evaluate the mobilization of HSC and HPC using AMD3100, Indomethacin, or the combination of AMD3100 plus Indomethacin.

(B) Mobilization of CFU-GM by vehicle or Indomethacin (50 µg, *bid sc*, 4 days) treatment alone (left panel). Mobilization of CFU-GM by a single administration of AMD3100 (5 mg/kg on day 5), or Indomethacin treatment plus AMD3100 (right panel) was determined. Data are expressed as Mean ± SEM, N=5 mice per group, each assayed individually. $P < 0.05$.

Figure 24

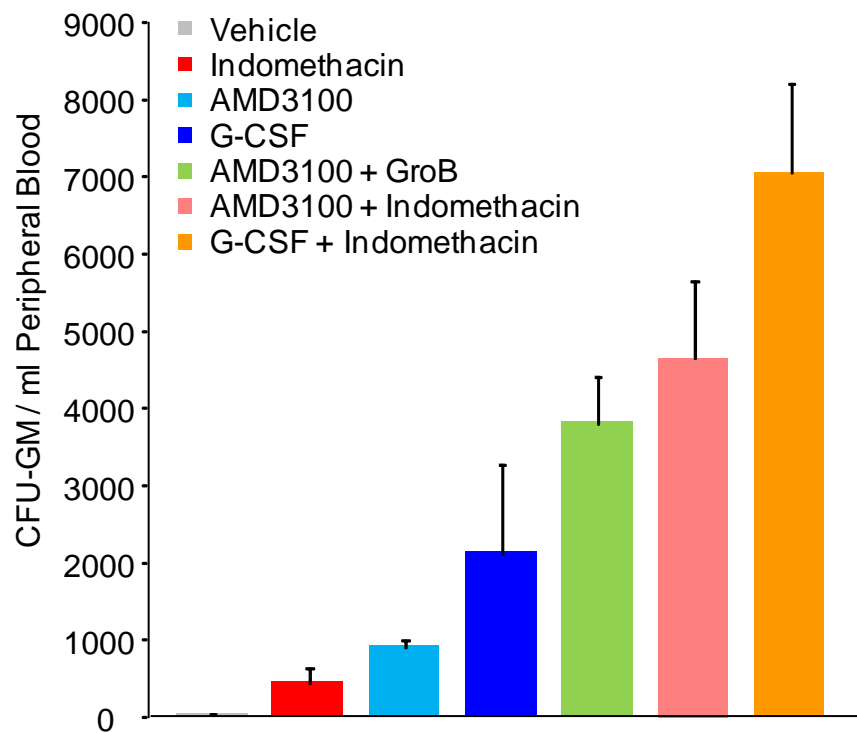


Figure 24. Enhancement in mobilization by NSAIDs is independent of mobilizing agent mechanism.

Mice were treated with vehicle, Indomethacin (50 µg, *bid sc*, 4 days), AMD3100 (5 mg/kg day 5), G-CSF (1 µg, *bid sc*, 4 days), AMD3100 plus GROβ (5 mg/kg and 20 mg/kg respectively, day 5), AMD3100 plus Indomethacin (Indomethacin 50 µg, *bid sc*, 4 days; AMD3100 5 mg/kg day 5), or G-CSF plus Indomethacin (1 µg and 50 µg respectively, *bid sc*, 4 days). Mobilization of CFU-GM was determined. Data are expressed as Mean ± SEM, N=4 mice per group, each assayed individually.

Enhanced mobilization requires inhibition of COX1 and COX2

Increases in HPC within the bone marrow by NSAID administration required inhibition of both COX1 and COX2 enzymes (Figure 19). To evaluate the optimal NSAID for facilitation of HSC and HPC mobilization, NSAIDs with relative COX1 to COX2 selectivity were evaluated in combination with G-CSF for their ability to mobilize CFU-GM. Administration of highly selective COX1 NSAIDs (SC-560 and Valeryl Salicylate) or highly selective COX2 NSAIDs (Celecoxib, Valdecoxib, NS-398) resulted in no significant enhancements in mobilization over G-CSF alone (Figure 25). However, administration of NSAIDs having dual COX1 and COX2 inhibition capabilities (Indomethacin, Aspirin, Ibuprofen or Meloxicam) resulted in significant enhancement of mobilization over G-CSF alone, in agreement with the earlier findings on bone marrow HPC expansive capabilities of NSAIDs. While dual COX/LOX inhibitors have been shown to inhibit migration, selectins, and leukocyte rolling and adhesion (Ulbrich et al., 2005; Zhou et al., 1996); administration of the COX/LOX inhibitor Licofelone showed no enhancements in mobilization over NSAIDs, further demonstrating that NSAID facilitation of mobilization is due to COX1 and COX2 inhibition, and not due to general eicosanoid inhibition. While Meloxicam inhibits both COX2 and COX1 at normal physiological doses (Kato et al., 2001; Shi and Klotz, 2008), when compared to other dual inhibitors, it has a reduced incidence of gastrointestinal (GI) discomfort (Ahmed et al., 2005) and inhibition of platelet aggregation (Rinder et al., 2002), adverse events usually associated with

NSAIDs. We believe that Meloxicam has an ideal clinical profile, and will be the NSAID of focus of the remainder of described studies.

Figure 25

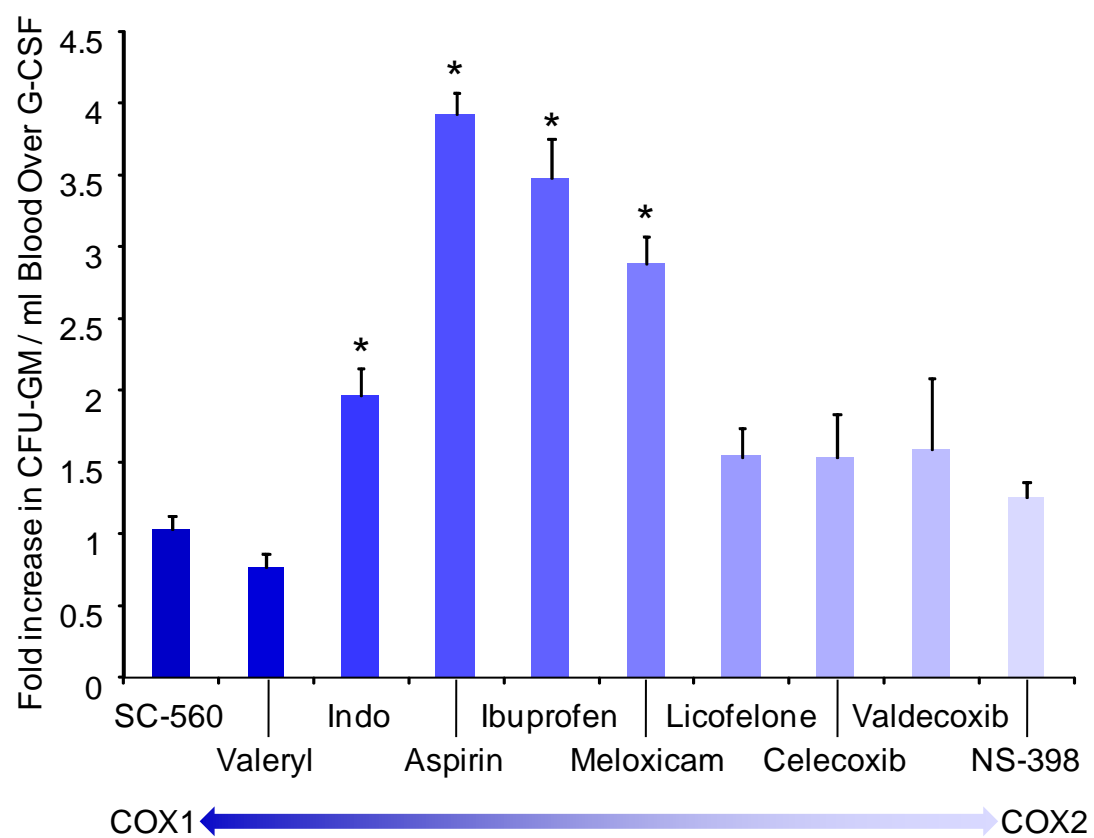


Figure 25. Enhancement in mobilization requires inhibition of COX1 and COX2.

Mice were mobilized by a 4 day regimen of G-CSF (50 µg/kg, twice daily) with co-administration of NSAIDs ranging in COX1 (left) and COX2 (right) selectivity: SC-560 (10 mg/kg, twice daily), Valeryl Salicylate (10 mg/kg, twice daily), Indomethacin (2.5 mg/kg, twice daily), Aspirin (50 mg/kg, twice daily), Ibuprofen (40 mg/kg, twice daily), Meloxicam (6 mg/kg, once daily), Licofelone (10 mg/kg, twice daily), Celecoxib (40 mg/kg, twice daily), Valdecoxib (10 mg/kg, twice daily) and NS-398 (10 mg/kg, twice daily). Data are expressed as fold change over G-CSF alone, Mean ± SEM, N=4 mice per group, each assayed individually.

*P<0.05.

NSAID co-administration with G-CSF facilitates mobilization of a superior hematopoietic graft with enhanced recovery and long-term repopulation

Analysis of mobilization facilitation by NSAIDs up to this point has relied primarily on functional HPC colony assays and immunophenotypic analysis of HSC and HPC by FACS. To determine if NSAIDs enhanced mobilization of HSC capable of reconstitution, donor mice were treated with G-CSF with or without NSAID for 4 days, and peripheral blood LDMC isolated and transplanted competitively into lethally irradiated congenic recipients. Analysis of CRU 12 weeks post-transplant demonstrated no significant increases in repopulating units (Figure 26), despite observed increases in CFU-GM, and SKL and SLAM SKL cells (Figure 21). Since we previously demonstrated that PGE₂ signaling enhances CXCR4 expression and homing and engraftment of HSC (Hoggatt et al., 2009), we hypothesized that NSAID administration, while increasing HPC in bone marrow and mobilization to peripheral blood, may be reducing CXCR4 expression on HSC and HPC, thus reducing the ability of NSAID facilitated grafts to home and engraft in recipients. To address this issue, donor mice were treated in a staggered regimen, such that mice still received a standard 4 day regimen of G-CSF and a 4 day regimen of Meloxicam, however the Meloxicam regimen was either administered concurrently with G-CSF, or started earlier such that it stopped 1 or 2 days before the end of the G-CSF regimen (Figure 27A), to allow for restoration of PGE₂ biosynthesis and signaling. Analysis of CXCR4 receptor expression on mobilized SKL cells demonstrated significant reductions

in CXCR4 expression when Meloxicam was concurrently administered, or administered with a 1 day stagger (Figure 27B), whereas, 2 day staggered administration resulted in restoration of CXCR4 receptor expression, when compared to G-CSF alone. To evaluate repopulating ability of these staggered and non-staggered NSAID facilitated grafts, we utilized a limiting-dilution competitive repopulation assay, transplanting LDMC from mobilized mice at 1:1, 2:1, 3:1 and 4:1 ratios with congenic WBM competitor cells into lethally irradiated mice. Analysis of chimerism 12 weeks later demonstrated significantly enhanced repopulation with grafts mobilized with staggered NSAID administration (Figure 28A) when compared to non-staggered administration, or G-CSF alone. In addition, analysis of CRU (Figure 28B) or LT-HSC frequency as calculated by Poisson distribution (Figure 29) demonstrated significantly enhanced long-term repopulating units in staggered NSAID facilitated grafts, when compared to non-staggered administration or G-CSF alone, with a 2 day stagger resulting in a 2.55 fold increase in LT-HSC frequency over the G-CSF mobilized graft ($P_0 = 4.82 \times 10^5$ and $P_0 = 1.23 \times 10^6$, respectively). These results clearly indicate that staggered NSAID co-administration with G-CSF can significantly increase the repopulating ability of G-CSF mobilized grafts.

Figure 26

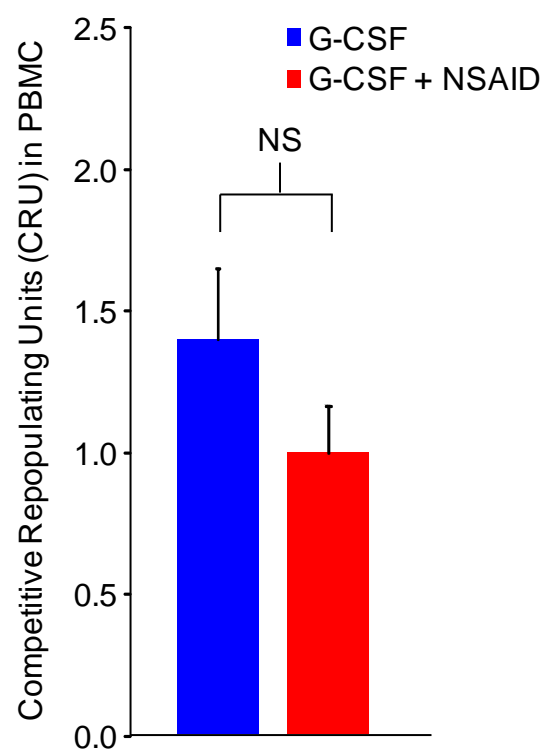


Figure 26. Non-staggered administration of G-CSF and NSAID results in no increase in CRU in the mobilized graft.

BoyJ mice were treated with G-CSF with or without NSAID for 4 days. On day 5, LDMC from peripheral blood were acquired and transplanted at a 1:1 ratio with C57Bl/6J WBM competitors into lethally irradiated C57Bl/6J recipient mice. Competitive repopulating units were calculated 3 months post-transplant (Mean \pm SEM). Data represent 2 pooled experiments, N=5 mice per group, per experiment, each assayed individually.

Figure 27

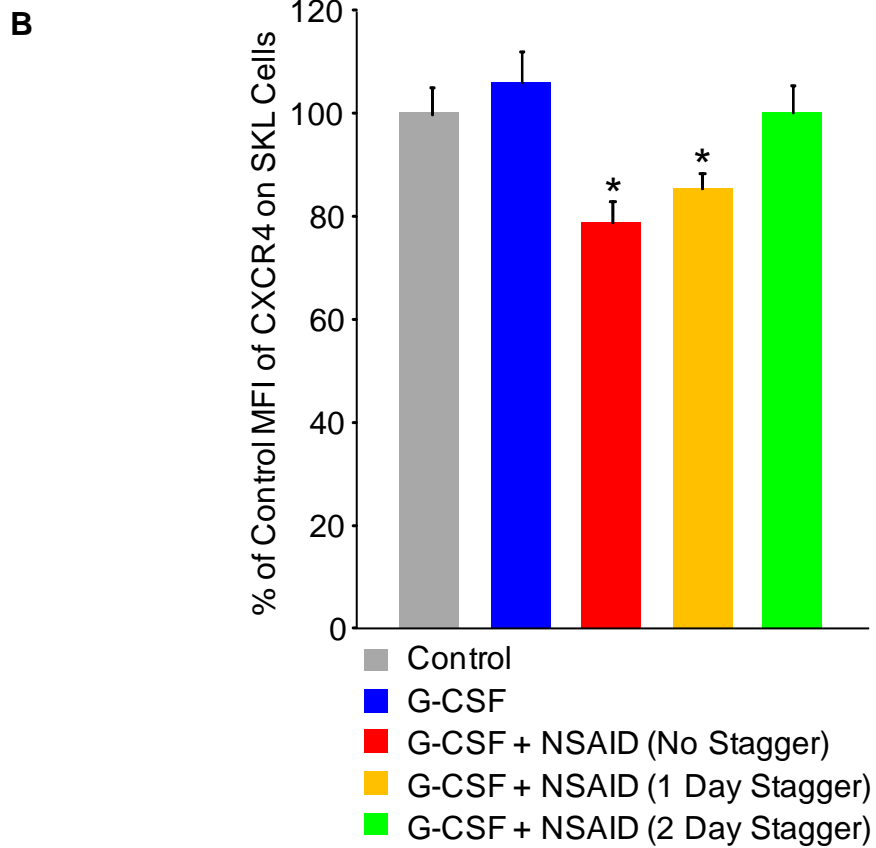
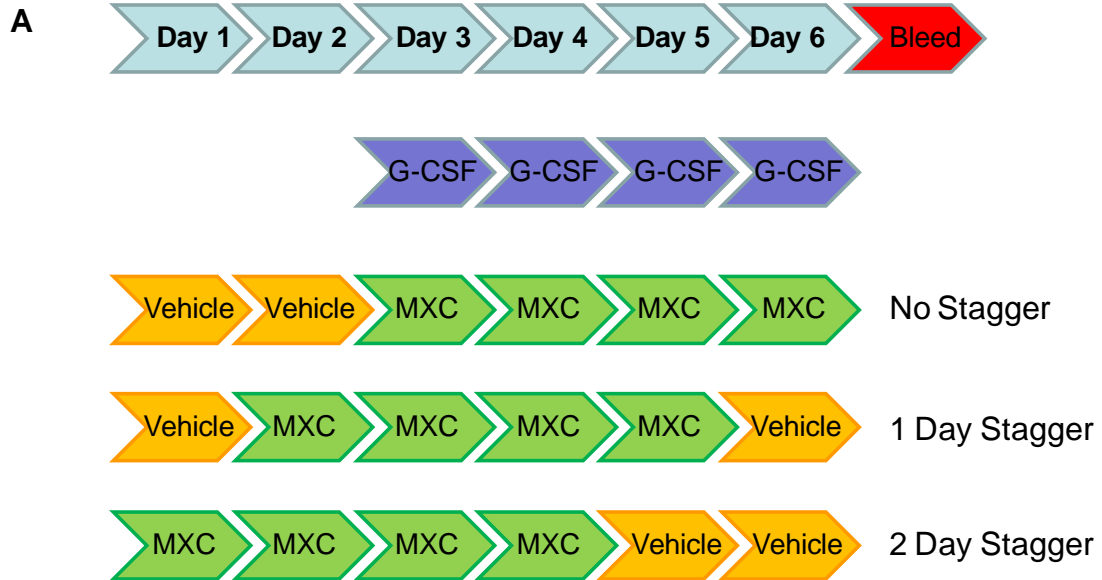


Figure 27. Staggered administration of NSAID with G-CSF restores CXCR4 expression on HSC.

(A) Schematic of staggered dosing regimens of G-CSF with Meloxicam (MXC).

A 4 day regimen of Meloxicam was either co-administered with G-CSF for 4 days (no stagger) or was staggered 1 or 2 days to allow for restoration of PGE₂ biosynthesis.

(B) Mice were bled and PBMC were stained for SKL and the CXCR4 receptor and evaluated by FACS. Data represent the percent of control mean fluorescence intensity (MFI) for CXCR4 expression on SKL cells, expressed as Mean \pm SEM, N=5 mice per group, each assayed individually. *P<0.05.

Figure 28

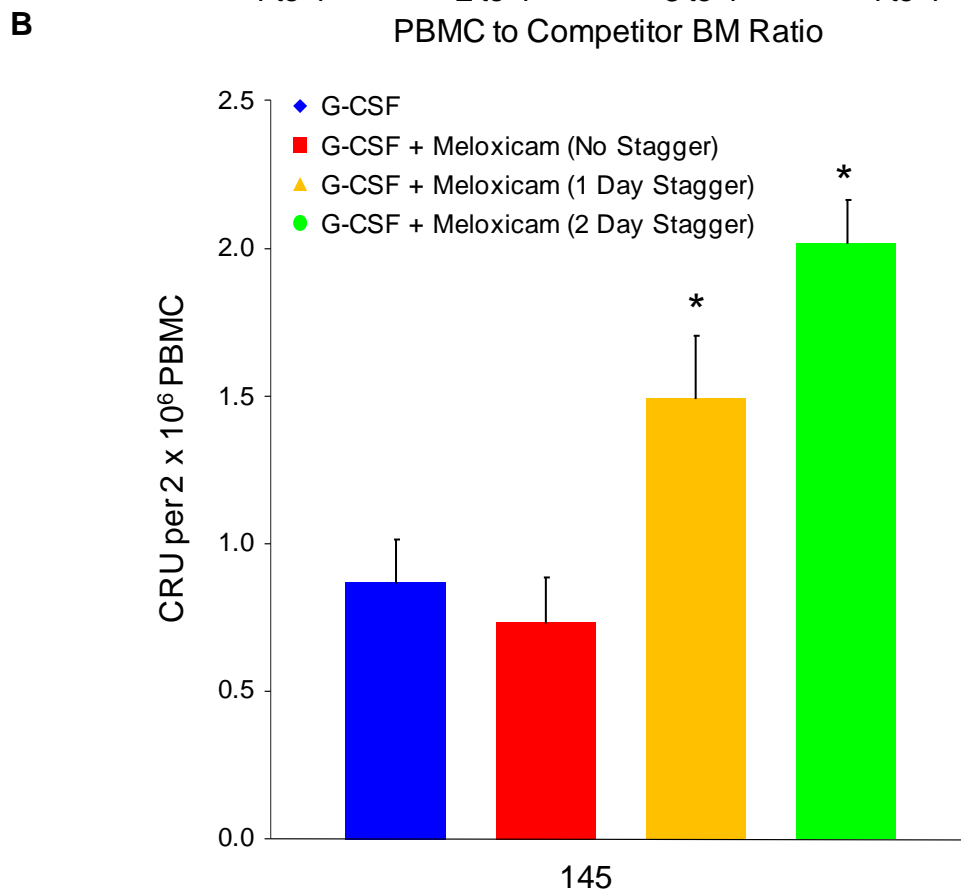
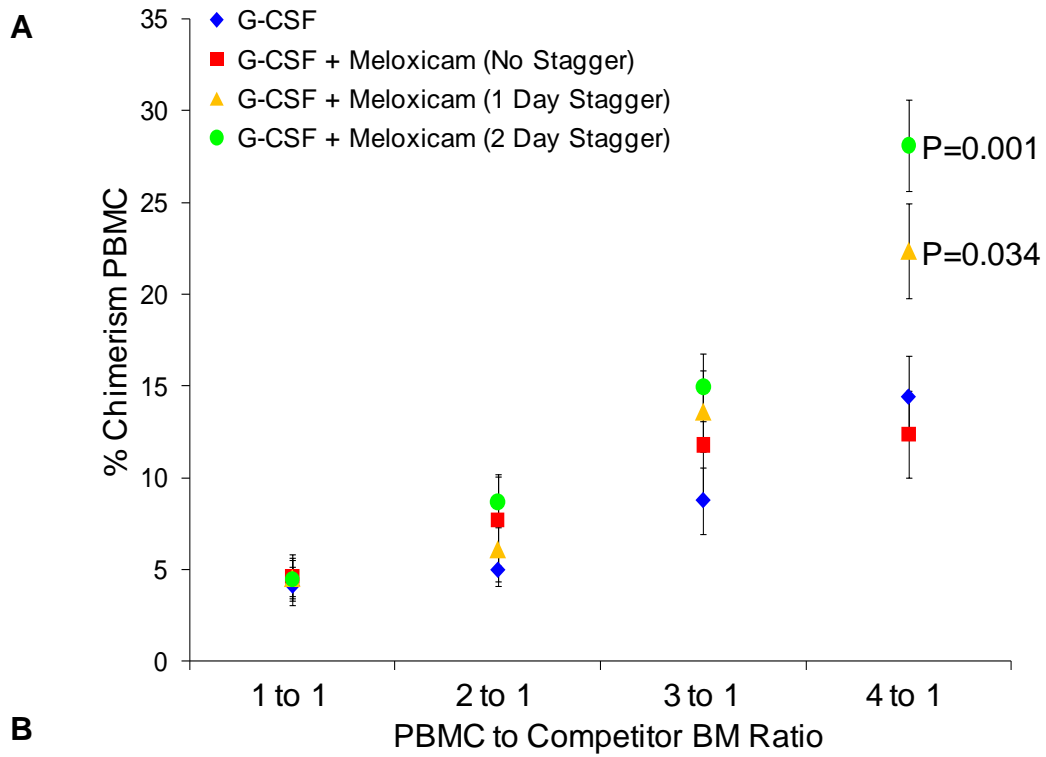


Figure 28. Staggered administration of NSAID with G-CSF enhances mobilized graft repopulating ability.

(A) BoyJ mice were treated with G-CSF with or without Meloxicam for 4 days with either no stagger, 1 day stagger, or 2 day stagger. On day 5, LDMC from peripheral blood were acquired and transplanted at a 1:1, 2:1, 3:1 or 4:1 ratio with 5×10^5 C57Bl/6J WBM competitors into lethally irradiated C57Bl/6J recipient mice. Chimerism at multiple donor competitor ratios is shown. Data are expressed as Mean \pm SEM, N=5-8 mice per group, each assayed individually.

(B) Shown are competitive repopulating units of mobilized grafts with or without staggered Meloxicam administration calculated from mice receiving the 4:1 ratio of LDMC to WBM competitors. Data are expressed as Mean \pm SEM, N=8 mice per group, each assayed individually. * $P < 0.05$.

Figure 29

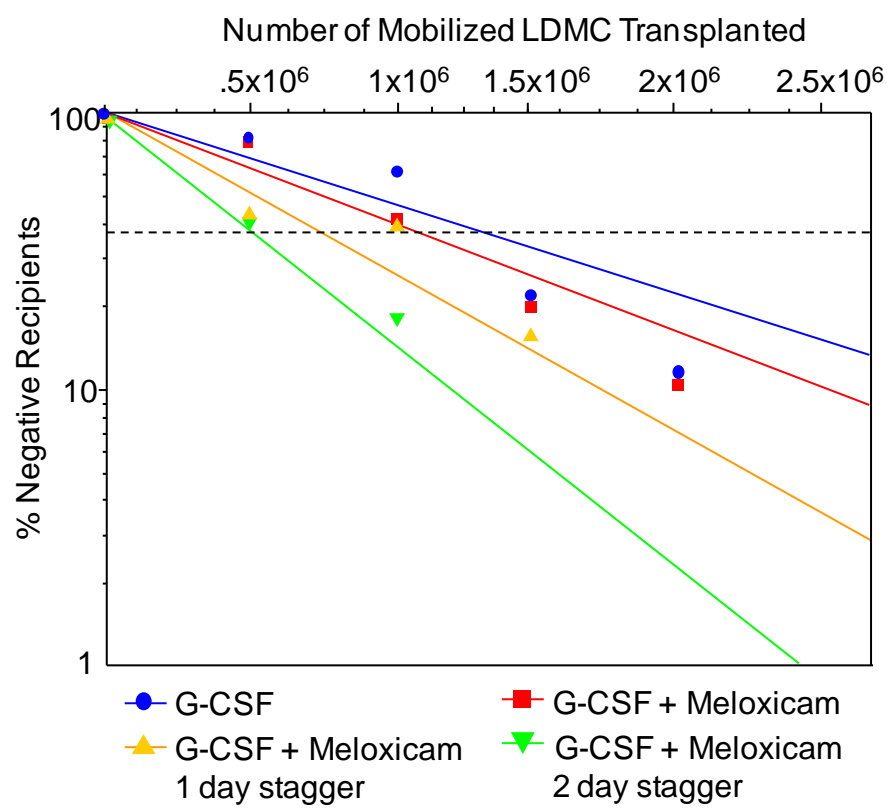


Figure 29. Staggered administration of NSAID with G-CSF enhances mobilized graft LTRC frequency.

Frequency analysis for G-CSF with or without staggered Meloxicam mobilized grafts, determined by Poisson statistics, at 12 weeks; $P_0 = 1.23 \times 10^6$ (G-CSF), $P_0 = 1.09 \times 10^6$ (G-CSF + Meloxicam), $P_0 = 7.27 \times 10^5$ (G-CSF + Meloxicam 1 day stagger), $P_0 = 4.82 \times 10^5$ (G-CSF + Meloxicam 2 day stagger).

NSAID facilitated grafts enhance hematopoietic recovery

Successful hematopoietic transplantation requires engraftment of HSC capable of lifelong blood reconstitution in recipients. In addition to long-term engraftment, early recovery of blood parameters, particularly neutrophils and platelets, is important to reduce the length of time of neutropenia and thrombocytopenia associated with myeloablative conditioning regimens and subsequent transplant related morbidity and mortality (Gerson et al., 1984; Pizzo, 1984; Taylor et al., 1989). Increasing the number of HSC and HPC contained within a hematopoietic graft has been demonstrated to reduce the time necessary for neutrophil and platelet recovery (Bittencourt et al., 2002), while the functional quality of the transplanted graft is also important (Graf et al., 2001). We evaluated the recovery kinetics of neutrophils and platelets in lethally irradiated mice that received transplants containing equivalent numbers of peripheral blood LDMC from donors mobilized with either G-CSF or a staggered G-CSF plus NSAID regimen. In mice receiving the G-CSF plus NSAID mobilized graft, ~4 day faster recovery of both neutrophils (Figure 30A) and platelets (Figure 30B) was observed and maintenance of neutrophil and platelet levels after full recovery was seen throughout 90 days for both transplant recipient groups. After 90 days, the mice were sacrificed and bone marrow transplanted competitively into lethally irradiated congenic recipients to evaluate LT-HSC. Secondary recipients receiving grafts from the G-CSF plus NSAID graft donors had significantly higher peripheral blood chimerism 16 weeks post-transplant

compared to G-CSF graft donors (Figure 31), demonstrating that increased early recovery of blood parameters of G-CSF plus NSAID mobilized grafts did not occur at the expense of long-term repopulation, but rather is coupled with increased long-term repopulation, suggesting that NSAID facilitated grafts are a superior transplantation product.

NSAIDs facilitate mobilization in non-human primates

To validate the NSAID enhancing effects seen in murine mobilization, 4 baboons were mobilized with 5 days of G-CSF or G-CSF plus Meloxicam, in a crossover design protocol, allowing for analysis of NSAID enhancement in individual baboons (Figure 32A). While individual baboons varied in response to mobilization regimens, peripheral blood CD34⁺ cells counts (2.73 ± 0.94 fold) and CFU-GM (4.58 ± 1.78 fold) were significantly higher in all 4 baboons treated with G-CSF plus NSAID co-administration compared to G-CSF alone (Figure 32B). In addition, administration of Meloxicam without G-CSF resulted in a rise in CD34⁺ cell counts (5.22 ± 0.61 fold) and CFU-GM (2.07 ± 0.12 fold) compared to baseline levels (Figure 33), consistent with findings of administration of NSAID alone in mice. These results clearly indicate that NSAID facilitation of PBSC mobilization, both with and without an additional mobilization agent, is successful in non-human primates.

Figure 30

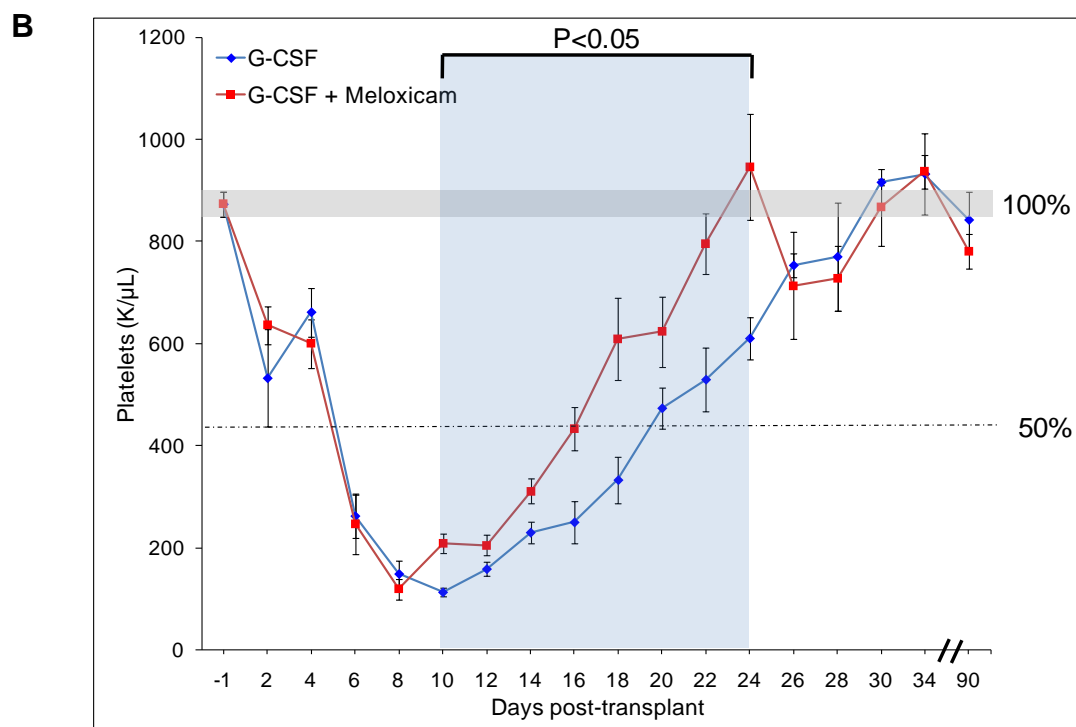
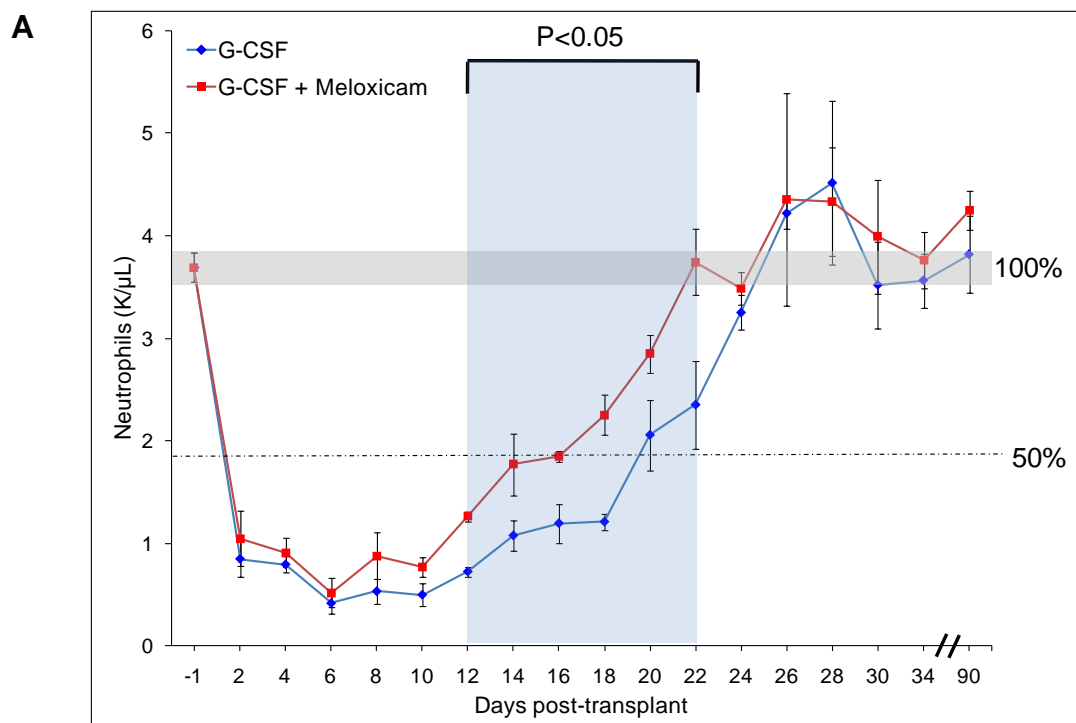


Figure 30. NSAID co-administration enhances neutrophil and platelet recovery capacity of G-CSF mobilized grafts.

Mice were mobilized with G-CSF or staggered administration of G-CSF plus Meloxicam and 2×10^6 PBMC were transplanted into lethally irradiated recipients.

(A) Neutrophils and (B) platelets in blood were enumerated with a Hemavet 950FS every other day in alternate groups of 5 mice until full recovery (compared to control subset). Data are expressed as Mean \pm SEM, N=10 mice per group, each assayed individually. $P < 0.05$ determined using a Student's T-test at each of the time points.

Figure 31

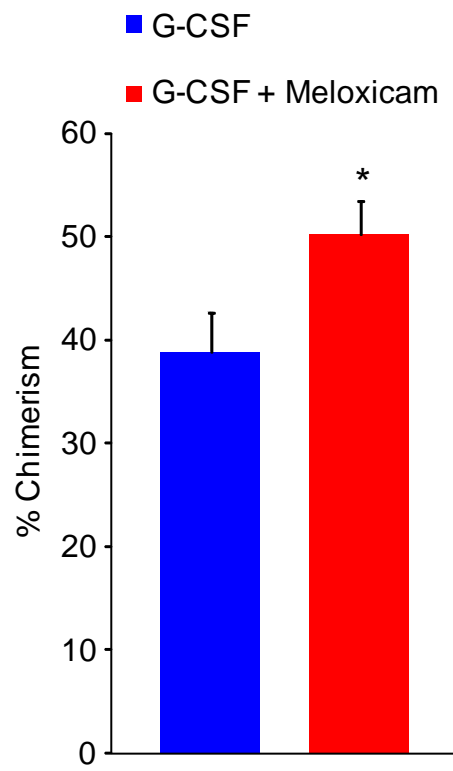
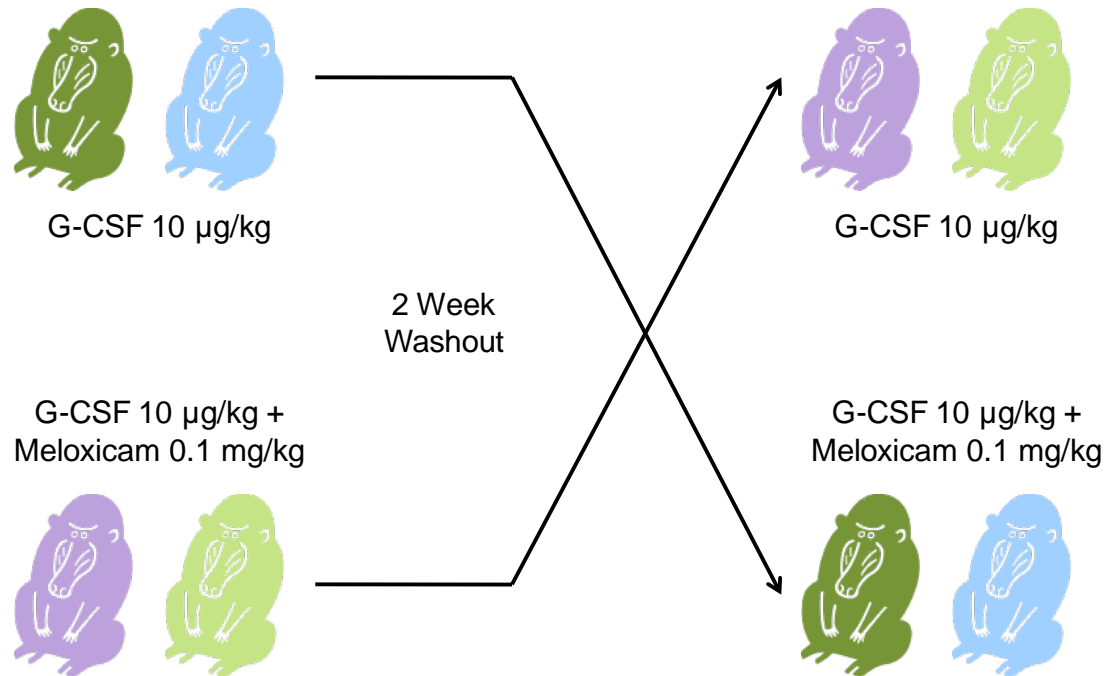


Figure 31. NSAID facilitated grafts retain long-term repopulating ability.

Mice were mobilized with G-CSF or staggered administration of G-CSF plus Meloxicam and 2×10^6 PBMC were transplanted into lethally irradiated recipients. Neutrophils and platelets in blood were enumerated every other day until full recovery. After 90 days, mice were sacrificed, bone marrow acquired, and transplanted at a 2.5:1 ratio with congenic competitors into lethally irradiated recipients. Peripheral blood chimerism at 16 weeks post-transplant is shown. Data are expressed as Mean \pm SEM, N=10 mice per group, each assayed individually. *P<0.05.

Figure 32

A



B

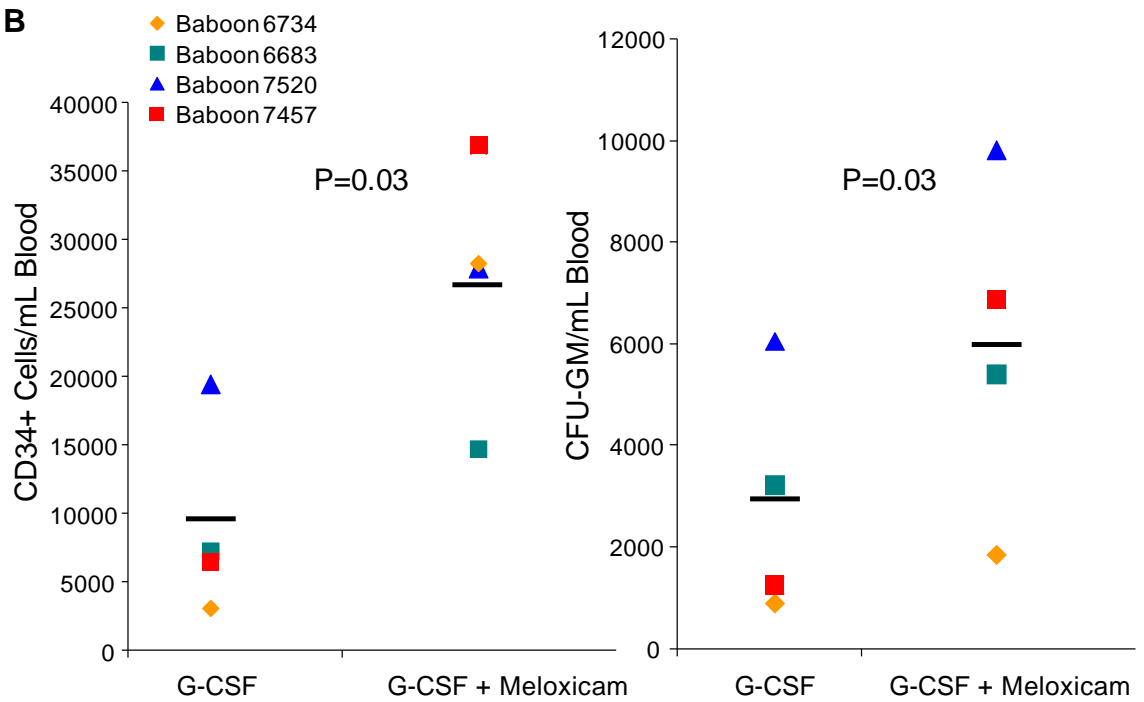


Figure 32. Enhanced mobilization in baboons with co-administration of Meloxicam with G-CSF.

(A) Schematic of baboon mobilization crossover design. Baboons were mobilized with G-CSF or G-CSF plus Meloxicam for five days. Blood was collected after treatment and the number of CFU-GM and CD34⁺ cells determined. Baboons were rested for 2 weeks, and then given the alternate treatment, allowing comparison of both treatment regimens within individual animals.

(B) CD34⁺ cells in PB were determined by FACS analysis (left panel). CFU-GM per ml of blood are shown for both treatment groups (right panel). The black bar represents the average CD34⁺ and CFU-GM values.

Figure 33

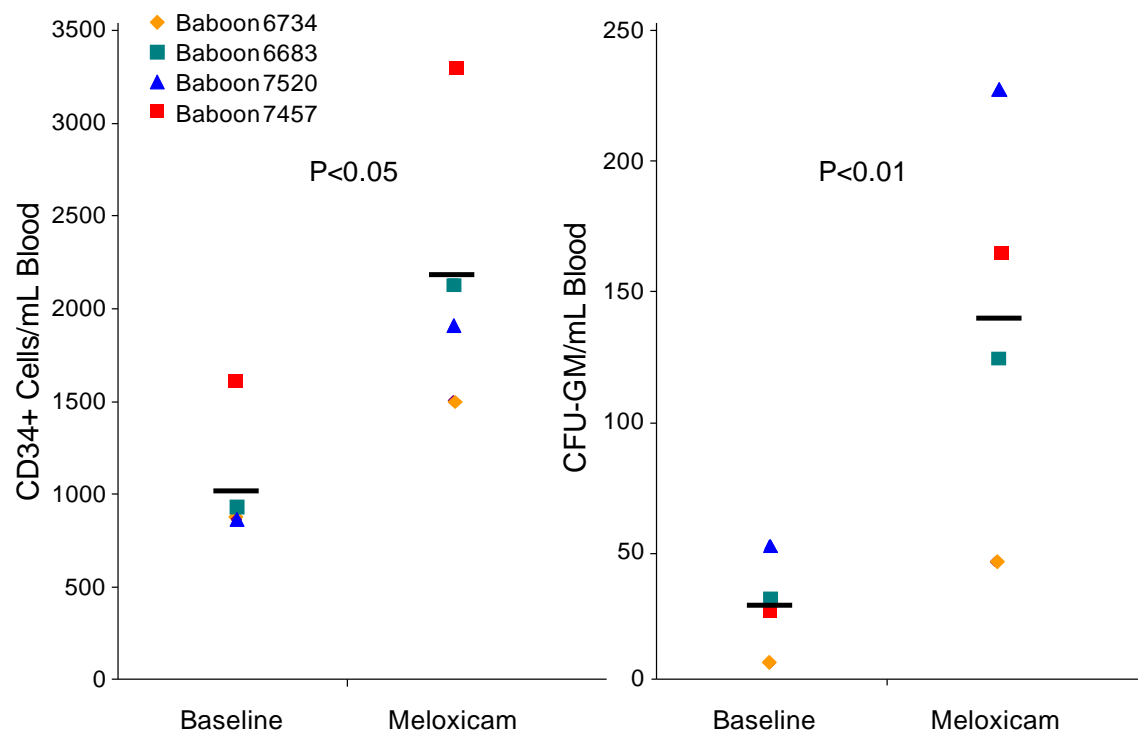


Figure 33. Meloxicam administration increases CD34⁺ cells and CFU-GM in peripheral blood in baboons.

Baboons were bled to establish baseline parameters, followed by a 5 day regimen of Meloxicam. CD34⁺ cells in PB were determined by FACS analysis (left panel). CFU-GM per ml of blood are shown for both treatment groups (right panel). The black bar represents the average CD34⁺ and CFU-GM values.

Enhancement in mobilization is mediated through a reduction in EP4 receptor signaling

Mobilization studies described thus far used NSAIDs to block PGE₂ biosynthesis, and clearly demonstrate enhancements in HSC and HPC mobilization. Since PGE₂ signals through four different receptors (EP1-4) (Breyer et al., 2001; Hull et al., 2004; Sugimoto and Narumiya, 2007; Tsuboi et al., 2002), we hypothesized that antagonism of one or more of these receptors would mimic the mobilization enhancing effects of NSAIDs. Mice were administered EP specific antagonists plus G-CSF, or EP specific agonists plus NSAID plus G-CSF, and CFU-GM mobilization to peripheral blood was assessed. Co-administration of EP4 antagonists (AH23848 and L-161,982) with G-CSF significantly enhanced mobilization over G-CSF alone, while an EP1-3 antagonist (AH6809) had no effect (Figure 34). Furthermore, when a selective EP4 agonist (L-902,688) was administered along with G-CSF plus NSAID, the NSAID enhancement in mobilization was abrogated, and to the same degree as dmPGE₂ co-administration, while the EP2 agonist (Butaprost) or EP1/3 agonist (Sulprostone) were unable to reduce the effects of NSAIDs, indicating that a lack of EP4 receptor signaling is responsible for NSAID enhanced HSC and HPC mobilization.

Using semisolid culture assays of mouse and human bone marrow cells, previous work showed that PGE₂ dose-dependently inhibits CFC proliferation (Pelus et al., 1979; Pelus et al., 1981), and preferentially inhibits monocyte-

committed CFC differentiation (Pelus et al., 1979; Pelus et al., 1981). These effects of PGE₂ were reproduced using db-cAMP or agents that increase cAMP or inhibit its metabolism (Kurland et al., 1977; Taetle and Koessler, 1980). Although the existence of multiple EP receptors was unknown at the time these studies were performed, new studies in our lab have utilized available pharmacological EP receptor selective agonists and antagonists to define the receptors involved in PGE₂ suppression of myelopoiesis (Table 5). As expected, EP1 and EP3 receptors were not involved, consistent with the fact that they do not link to an increase in cAMP, as depicted previously in Figure 3. The EP2 receptor agonist Butaprost was without activity whereas the weak EP4 agonist 1-hydroxy PGE₁ showed inhibitory activity, albeit at 2 log higher concentration, suggesting that EP4 likely mediates the inhibitory activity of PGE₂. Dose curve analysis in the presence of the selective EP4 antagonist AH23848 showed antagonism of PGE₂ with Schild plot analysis clearly showing that the EP4 antagonist was a competitive inhibitor of the PGE₂ effect. A log ratio of agonist to antagonist of -5.77 was determined on CFU-M, which is consistent with the antagonist ratio of AH23848 on other cell types (Smith et al., 1994). These results coupled with our *in vivo* data, suggest a model in which a lack of EP4 signaling drives progenitor expansion, possibly elucidating one of the mechanisms responsible for increased CFU-GM found in blood and marrow post-NSAID treatment.

Figure 34

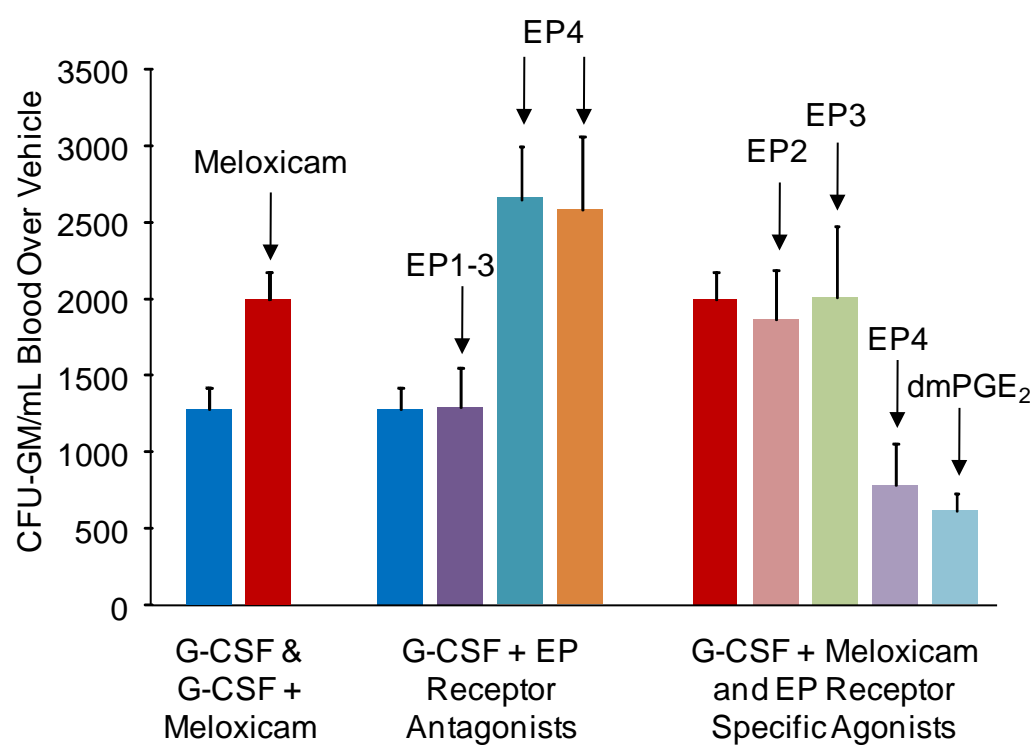


Figure 34. Enhanced mobilization mediated through a reduction in EP4 receptor signaling.

Mobilization enhancement with G-CSF plus Meloxicam (red bar) over G-CSF alone (blue bar). Enhanced mobilization over G-CSF is not recapitulated with an antagonist for EP receptors 1, 2 and 3 (AH6809), but is enhanced with 2 different EP4 receptor antagonists (AH23848 and L-161,982). Meloxicam enhancement of G-CSF mobilization is not blocked by co-treatment with an EP2 agonist (Butaprost) or an EP1/3 agonist (Sulprostone), but is blocked by an EP4 agonist (L-902,688), which mimics the effects of dmPGE₂.

Table 5

| Inhibition of CFU-M | | | |
|--|---------------------|-----------------------|--------|
| Compound | Agonist Specificity | IC ₅₀ (nm) | Ratio |
| PGE ₂ | EP1, EP2, EP3, EP4 | 2.37 ± 0.3 | 1 |
| dmPGE ₂ | EP2, EP3, EP4 | 3.1 ± 0.9 | 2.1 |
| 17-phenyl trinor PGE ₂ | EP1, EP3 | 1392 ± 383 | 587 |
| Butaprost | EP2 | 89,100 ± 2145 | 37,595 |
| Sulprostone | EP3, EP1 | 6990 ± 349 | 2949 |
| 11-deoxy PGE ₂ | EP2, EP3, EP4 | 11.5 ± 5 | 4.8 |
| 1-hydroxy PGE ₁ | EP4 [weak] | 147 ± 28 | 62 |
| PGE ₂ | | 2.4 ± 0.3 | 1 |
| + 5µM AH23848 | EP4 antagonist | 10.6 ± 0.2 | 4.5 |
| + 10µM AH23848 | “ ” | 14.6 ± 0.6 | 6.2 |
| + 30µM AH23848 | “ ” | 36.8 ± 4.5 | 15.5 |
| + 60µM AH23848 | “ ” | 92.1 ± 14.6 | 38.9 |
| Schild Plot Kb 1.69x10 ⁻⁶ M: R ² = 0.95 A/A'=-5.57: = Competitive Antagonist | | | |

(Work performed by Dr. Louis Pelus)

Table 5. Blockade of PGE₂ inhibition of CFU-M by EP4 antagonism

Mouse bone marrow cells (5×10^4 /plate) were cultured in 0.3% agar in supplemented McCoy's 5A medium with 10% HI-FBS and 10 ng/ml recombinant mouse macrophage colony-stimulating factor (M-CSF) (R&D Systems). Serial log dilutions of PGE₂ or EP receptor agonists over the dose range of 10^{-5} - 10^{-12} M were added directly to the bottom of the dishes prior to plating as previously described (Pelus et al., 1979; Pelus et al., 1981). For antagonist studies, marrow cells were incubated with PGE₂ or PGE₂ plus EP receptor antagonists in suspension culture for 30 minutes prior to plating. All plates were cultured for 7 days at 37 °C, 5% CO₂, 5% O₂ in air and total colonies per plate enumerated. IC₅₀ values were calculated based on control plates receiving M-CSF plus vehicle (0.01% ETOH). Data are expressed as Mean \pm SEM from 3-12 complete log dilution experiments for each compound. The IC₅₀ of authentic PGE₂ was set to a value of 1 and relative potencies of agonist or antagonist compounds calculated accordingly.

Reduced bone marrow osteoblasts and increased myelopoiesis in NSAID treated mice

Niche osteoblasts have been shown to regulate hematopoiesis (Arai et al., 2004; Calvi et al., 2003; Visnjic et al., 2004; Zhang et al., 2003) and are a major source of PGE₂ (Chen et al., 1997; Miyaura et al., 2003; Raisz et al., 1979) and other cytokines. Osteoblasts express all 4 EP receptors (Suzawa et al., 2000), with EP4 linked to regulation of osteoblast pool size (Shamir et al., 2004; Weinreb et al., 2006; Yoshida et al., 2002). Conversely, inhibition of PGE₂ synthesis with NSAIDs suppresses osteoblast proliferation and induces apoptosis (Chang et al., 2005; Kellinsalmi et al., 2007; Li et al., 2010). Since osteoblasts are an important component of the hematopoietic niche that maintains HSC, disruption of this niche can lead to HSC mobilization, which has been suggested as a possible mechanism for the mobilizing effects of G-CSF (Kollet et al., 2006). To begin to evaluate if the 4 day regimen of NSAIDs affects the hematopoietic niche and endosteal osteoblasts, femurs from vehicle and NSAID treated mice were isolated, fixed, sectioned and stained with hematoxylin and eosin (H&E). Femurs from NSAID treated mice exhibited a marked increase in myelopoiesis with full maturation, and a decrease in erythropoiesis (Figures 35 A,B). While osteoblasts were still present, there was a less distinct layer lining both bony trabeculae and cortical bone in the diaphyseal (Figures 35 C,D) and epiphyseal (Figures 35 E,F) regions of the femur. No overt changes were seen in total cellularity, megakaryopoiesis, or trabeculae morphology.

Figure 35

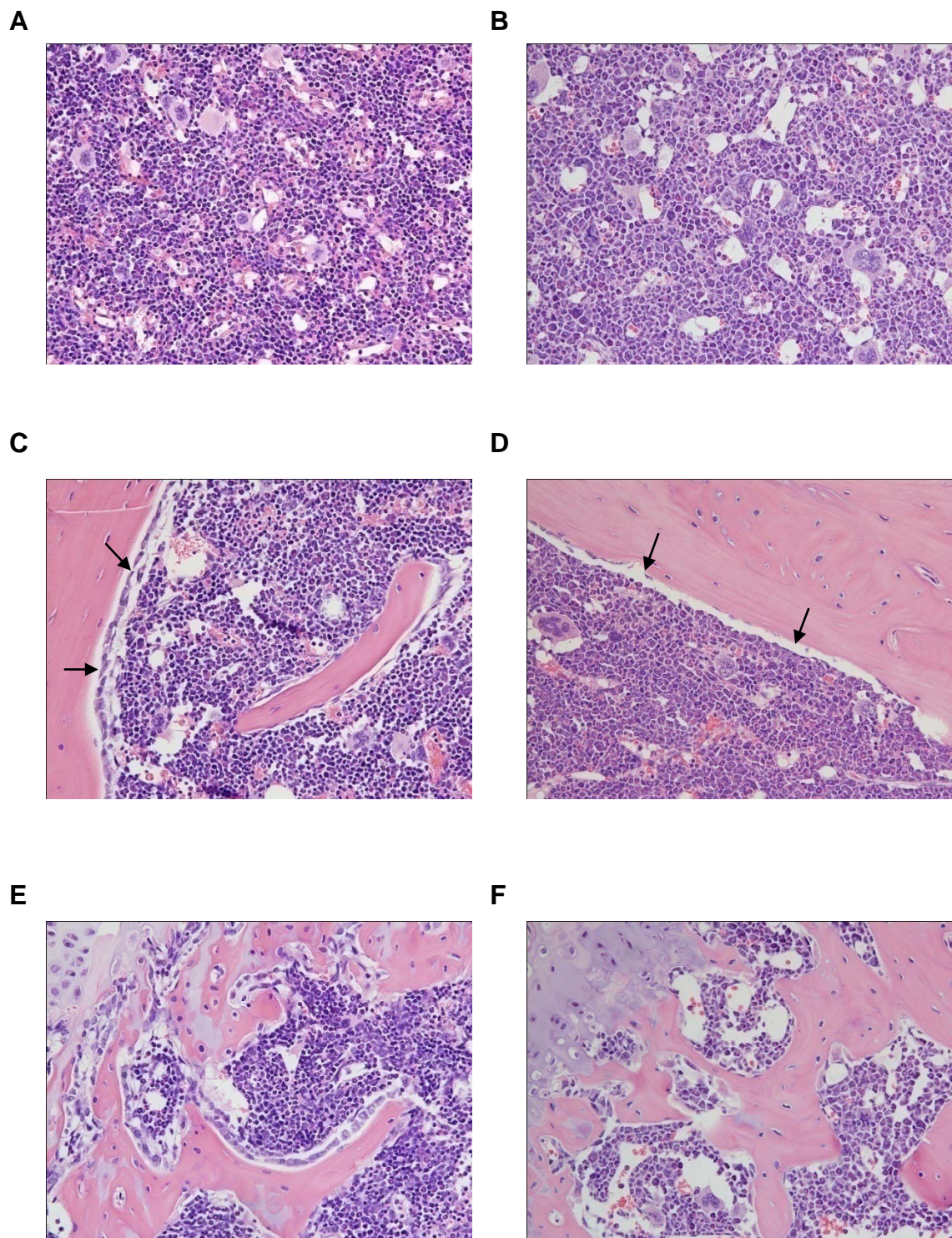


Figure 35. NSAIDs increase myelopoiesis and reduce osteoblasts in the bones of treated mice.

Mice were treated with a 4 day regimen of vehicle or NSAIDs, femurs were acquired, sectioned and H&E stained. Shown is the marrow from (A) control and (B) NSAID treated mice, showing increased myelopoiesis and reduced erythropoiesis after treatment.

(C) Control mice maintained normal osteoblast lining of bone (arrows) in the diaphyseal region of the femur, while (D) NSAID treated mice showed a less distinct layer of osteoblasts.

(E) Control mice also had normal osteoblast lining within the epiphyseal region of the femur, while (F) NSAID treated mice showed a less distinct layer of osteoblasts.

400X magnification

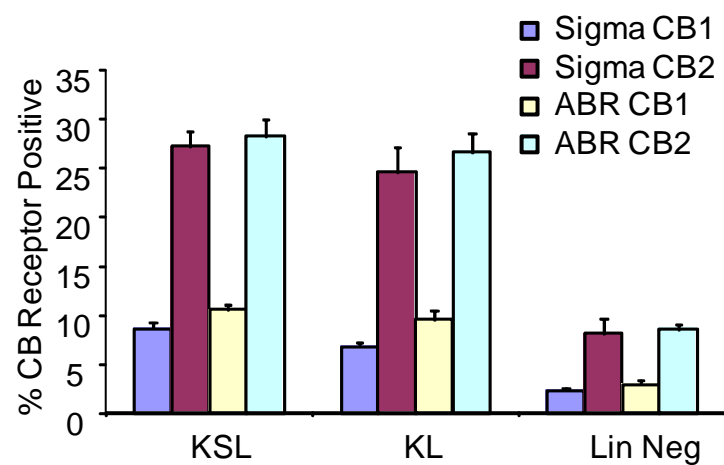
Cannabinoid receptor agonism mobilizes HPC

As was discussed in Chapter 1, the eicosanoid system consists of numerous signaling pathways, which have the capacity to complement or antagonize each other. Recent evidence suggests that cannabinoids, via the CB₂ receptor, inhibit SDF-1 α induced and CXCR4 mediated chemotaxis of Jurkat T-cells (Ghosh et al., 2006) and activated human derived peripheral T-cells (Coopman et al., 2007). In addition, cannabinoid receptor transfected hematopoietic cell lines migrate in response to the endogenous cannabinoid, 2-AG (Jorda et al., 2002). Cannabinoids have been reported to affect matrix metalloproteinase-9 (MMP-9) production (Rosch et al., 2006) in a number of cell lines and can modulate neutrophil function (Alberich et al., 2004; Kurihara et al., 2006). Moreover, the endogenous cannabinoid, anandamide, can be a synergistic growth factor for hematopoietic cells (Valk et al., 1997). Taken together, these data suggest that cannabinoids may play a role in PBSC mobilization, through interference in the SDF-1 α /CXCR4 axis and/or release of MMP-9, which can degrade niche tethering components (McQuibban et al., 2001) thereby increasing the HSC/HPC pool, or through undefined mechanisms.

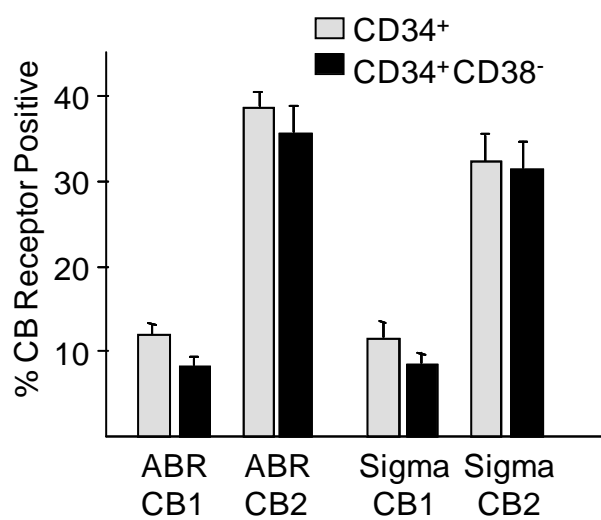
The presence of CB receptors on HSC and HPC has been controversial and earlier reports failed to demonstrate receptor expression on murine HPC (Jorda et al., 2002), likely due to issues of antibody specificity. Using two different recently developed anti-CB₁ and CB₂ polyclonal antibodies, we found that both the central CB₁ and peripheral CB₂ receptors are expressed on murine

Figure 36

A



B



C

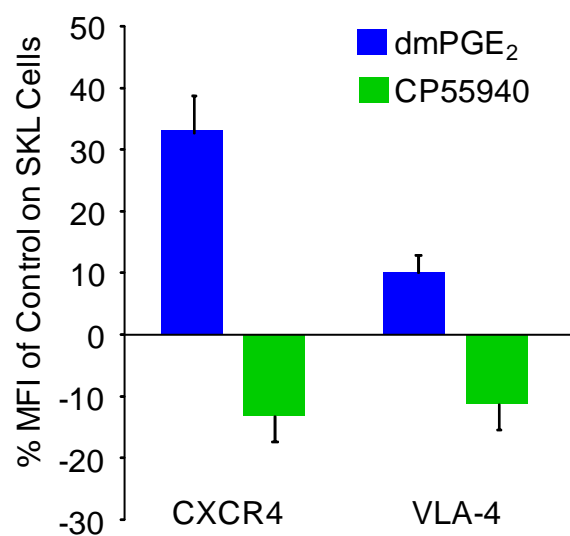


Figure 36. HSC and HPC express CB receptors and CB agonism decreases CXCR4 and VLA-4.

(A) Using two different antibodies for cannabinoid receptor expression (Sigma Aldrich and Affinity BioReagents (ABR)) mouse bone marrow cells were stained for SKL and the proportion of phenotyped cells expressing CB₁ and CB₂ determined. Data are expressed as Mean \pm SEM, N=5 mice, each assayed individually.

(B) Human CD34⁺ and CD34⁺ CD38⁻ UCB cells were stained with 2 different antibodies for CB₁ and CB₂ receptors. Data are Mean \pm SEM, N=3-5 UCB samples, each assayed individually.

(C) Mouse Lin^{neg} bone marrow was treated with dmPGE₂, the cannabinoid agonist CP55940, or vehicle control. Sixteen hours post-treatment, cells were stained for CXCR4 receptor or VLA-4, and SKL phenotypic markers, and % MFI compared to control treated cells determined. Data are Mean \pm SEM, N=5 mice, each assayed individually.

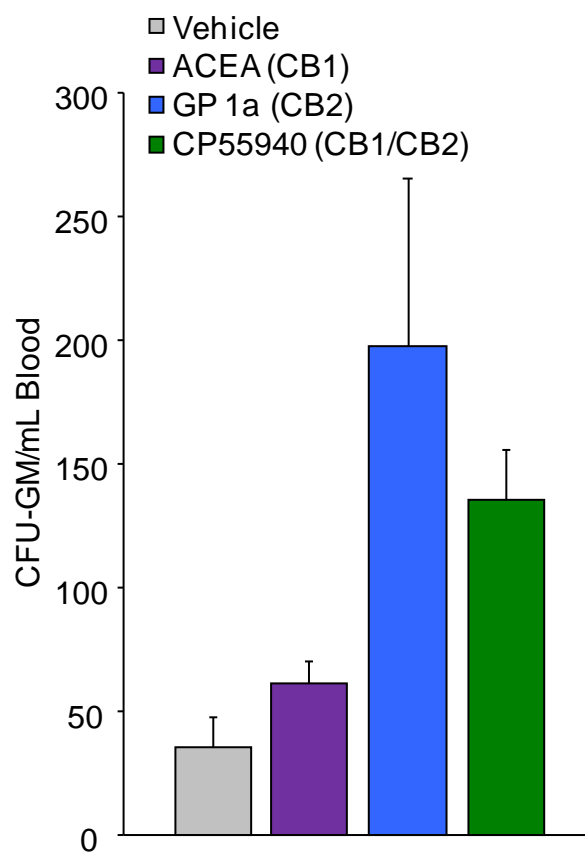
KL and SKL cells (Figure 36A), and on human CD34⁺ and CD34⁺ CD38⁻ cells (Figure 36B). The peripheral CB₂ receptor was the predominate receptor on murine and human HSC and HPC. In addition, we utilized the dual CB₁ and CB₂ agonist CP55940 (Thomas et al., 1998) in *in vitro* assays and examined expression of CXCR4 and the adhesion molecule VLA-4 on SKL cells, compared to cells treated with dmPGE₂. As we have previously reported, dmPGE₂ increased expression of CXCR4 on treated SKL cells (Figure 36C), and also increased expression of VLA-4. In contrast, SKL cells treated with cannabinoids demonstrated reduction in both receptors, suggesting that cannabinoids may be able to facilitate un-tethering of HSC and HPC from bone marrow niches.

To determine the effects of cannabinoid receptor agonists on HPC mobilization, BALB/c mice were treated with single injections of the CB₁ selective agonist ACEA (Pertwee, 1999), the CB₂ selective agonist GP1a (Murineddu et al., 2006), or the dual CB₁ and CB₂ agonist CP55940 and CFU-GM in peripheral blood was quantitated after 2 hours. Single administration of the CB₂ selective agonist GP1a and the dual CB₁/CB₂ agonist CP55940 significantly mobilized CFU-GM to peripheral blood (Figure 37A). The CB₁ selective agonist ACEA had only marginal mobilizing activity. In separate groups of mice, combination mobilization with G-CSF and the dual CB₁/CB₂ agonist CP55940 was evaluated. Mice were mobilized with a standard 4-day regimen of G-CSF alone, G-CSF plus a daily dose of CP55940, or the G-CSF regimen plus a single dose of CP55940 administered on day 5, 16 hours after the last dose of G-CSF and 2 hours prior to

sacrifice. The addition of CP55940 co-administered daily with G-CSF did not produce a significant increase in mobilization, however, the addition of a single dose of CP55940 on day 5 after the 4-day G-CSF regimen, ~16 hours after the last dose of G-CSF significantly increased CFU-GM mobilization compared to G-CSF alone (Figure 37B). The lack of effects of daily CP55940 may reflect timing or GPCR desensitization. These data suggest that CB receptor ligation, particularly CB₂, rapidly but transiently mobilizes CFU-GM, and that CB receptor activation enhances mobilization by G-CSF, likely by an effect on inhibition of CXCR4 signaling, or reductions in integrin adherence, since the kinetics of mobilization are in close alignment with the kinetics of mobilization by the CXCR4 antagonist AMD3100 and/or VLA-4 inhibitors.

Figure 37

A



B

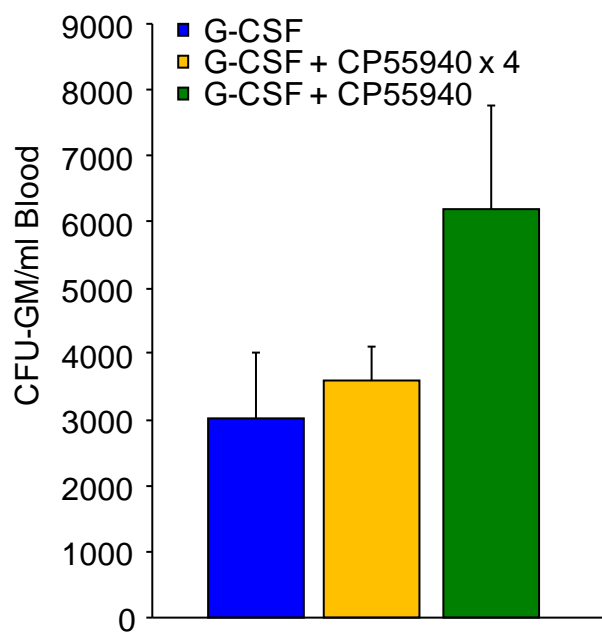


Figure 37. Cannabinoid agonism through the CB₂ receptor rapidly and synergistically mobilizes HPC.

(A) CFU-GM mobilization 2 hours post single administration of 10 mg/kg ACEA, 5 mg/kg GP1a or 10 mg/kg CP55940. Data are expressed as Mean \pm SEM, N=3 mice per group, each assayed individually.

(B) Mobilization by G-CSF, G-CSF + CP55940 (10 mg/kg) once daily for 4 days, or G-CSF once daily for 4 days, followed by a single dose of CP55940 on day 5. Data are expressed as Mean \pm SEM, N=3 mice per group, each assayed individually.

Discussion:

The inhibitory effects of PGE₂ on myelopoiesis have been well documented (Gentile and Pelus, 1988; Kurland et al., 1978; Kurland et al., 1979; Pelus et al., 1979; Pelus et al., 1981; Pelus et al., 1983; Pelus et al., 1988; Pelus and Gentile, 1988) though it is clear that its role in regulation of overall hematopoiesis is more complex. While PGE₂ signaling is inhibitory to CFU-M colony formation, it actually stimulates BFU-E and CFU-GEMM formation (Lu et al., 1984; Lu et al., 1986; Lu et al., 1987; Rossi et al., 1980), and we and others have recently reported positive effects on more immature HSC (Hoggatt et al., 2009; North et al., 2007). New data from our laboratory also indicate positive effects of PGE₂ on common dendritic progenitors (CDPs), and reductions in dendritic cell formation from these CDPs as a result of NSAID administration (Singh et al., 2009). We report here that inhibition of PGE₂ biosynthesis, and as a consequence, EP receptor signaling, results in an increase in HPC, both in bone marrow and in peripheral blood. Intriguingly, analysis of CFU-GM colonies from G-CSF mobilized mice compared to G-CSF plus NSAID mobilized mice show a marked shift in monocytic and multi-centric colony formation, which possibly indicates preferential expansion of an altered HPC pool and/or egress of a subset of HPC. Further detailed analysis of the exact phenotype and proportions of HPC present in bone marrow and blood following NSAID administration are currently ongoing.

It is important to note that in *in vitro* and *in vivo* assays, PGE₂ does not act alone but along with accompanying cytokines and growth factors. In response to stimuli, PGE₂, and its related eicosanoids, are almost instantly produced from already existing pools of phospholipids and enzymes and then are rapidly degraded. It is tempting to suggest a model in which PGE₂ and other eicosanoids are produced to rapidly shift fate decisions of stem and progenitor cells to readily respond to a particular challenge, followed by rapid restoration of homeostasis through metabolism of the eicosanoids, and subsequent loss of signaling. Isolation of single HSCs and HPCs, followed by *in vitro* tracking of fate decisions, which has recently been reported (Wu et al., 2007), may help to elucidate the fate decisions enacted by PGE₂ and other eicosanoids.

We have tested a broad panel of NSAIDs for their ability to expand HPC in bone marrow and to facilitate mobilization to peripheral blood. In both cases, only NSAIDs that inhibit both COX1 and COX2 had the ability to increase HPC and facilitate mobilization. These findings likely indicate that maximal reduction of PGE₂ signaling is needed to initiate progenitor expansion and that maintenance of activity of one of the COX enzymes and its resulting PGE₂ synthesis and signaling reduces/eliminates HPC expansion. The bulk of our described studies have utilized the NSAID Meloxicam, which was as effective as Indomethacin, Aspirin and Ibuprofen in facilitating mobilization. While Meloxicam is generally regarded as a COX2 selective NSAID, it is an efficient COX1 inhibitor (Kato et al., 2001; Shi and Klotz, 2008) at normal physiological doses. However,

Meloxicam shows reduced incidence of GI discomfort (Ahmed et al., 2005) and inhibition of platelet aggregation (Rinder et al., 2002), adverse events usually associated with dual COX inhibitors. Reduced inhibition of platelet aggregation is particularly noteworthy in the context of mobilization, which would employ central catheter lines for apheresis. We believe that Meloxicam has an ideal clinical profile for future translation into the clinic for PBSC transplantation.

As was discussed and depicted (Figure 2) in Chapter 1, regulation of eicosanoids is a complex process that employs a delicate balance of available substrate (Arachidonic Acid released by cPLA₂) and available enzymes (COX, LOX, FAAH, etc.). Disruptions of any of these strands of the “eicosanoid web” are likely to send ripple effects throughout the broader system. Numerous reports have begun to characterize effects of eicosanoids that are actually mediated by altering signaling/production of another class of eicosanoids (Ates et al., 2003; Lee et al., 2009; Massi et al., 2008; Stengel et al., 2007); for example, some effects of an NSAID can result from endocannabinoid signaling, or the effect of PGE₂ can result from reduction in LTB₄ signaling, etc. In our studies, we have blocked the biosynthesis of PGE₂ through inhibition of COX enzymes. However, these NSAIDs do not have direct effects on cPLA₂ release of Arachidonic Acid; therefore, the substrate is available for other enzymes like LOX or FAAH, and recent evidence demonstrates that this shunting of eicosanoid synthesis does occur as a result of NSAID administration (Duffield-Lillico et al., 2009). A condition known as “Aspirin induced asthma” is the result of increased

leukotriene production as a result of COX inhibition eicosanoid synthesis shunting (Park et al., 2010; Szczeklik and Sanak, 2006).

Our studies have utilized both a LOX inhibitor and a dual COX/LOX inhibitor and have found no effects on HPC mobilization by blocking leukotriene synthesis. These results, coupled with the findings that specific EP receptor antagonism can mimic the effects of NSAIDs and that co-treatment with dmPGE₂ or EP4 agonist with NSAID blocks the enhanced mobilization effect, strongly suggest that the NSAID facilitation of mobilization is specific to the COX-PGE₂ pathway; although we cannot completely rule out secondary effects due to eicosanoid shunting and cross-talk. Our results demonstrating mobilization by cannabinoid receptor agonism possibly suggest a role for this class of eicosanoids in hematopoietic trafficking. In light of our findings and those of others, it is clear that investigation of the role of eicosanoids in hematopoietic regulation should be cognizant of the system in its entirety, and it is highly likely that future therapeutic strategies that target multiple points of intervention in the eicosanoid pathway will be more effective; a proof of concept exists in the treatment of Aspirin induced asthma with leukotriene antagonists (Park et al., 2010).

Mobilization by the hematopoietic growth factor G-CSF and the chemokine CXCR4 receptor antagonist AMD3100 are significantly enhanced by NSAID co-treatment, which suggests that enhancement is independent of the mobilization mechanisms of the mobilizing agent. A small amount of mobilization is also seen

in both mouse and baboon after administration of NSAID alone, the implications of which are further discussed in Chapter 5. Extensive limiting dilution transplant analysis, assays monitoring neutrophil and platelet recovery, and secondary transplantation analysis, clearly indicate that NSAIDs facilitate mobilization of a hematopoietic graft with superior functional activity compared to the graft mobilized by G-CSF alone. Intriguingly, we found that despite increases in functionally and phenotypically defined HSC in peripheral blood when NSAIDs were administered concurrently with G-CSF, these parameters did not result in enhanced functional activity when analyzed in primary transplantation studies. We have previously reported (in Chapter 2 and (Hoggatt et al., 2009)) that PGE₂ signaling enhances HSC CXCR4 receptor expression and homing/engraftment to the bone marrow. This implies that loss of PGE₂ signaling in the marrow microenvironment might reduce CXCR4 expression, which we subsequently confirmed, and provides an explanation for the lack of benefit seen with NSAID mobilized PBSC graft despite containing increased HSC number. To compensate for reduced CXCR4 expression, we developed a novel staggered dosing regimen, which maintains the enhancements in HSC and HPC mobilization, but also allows for restoration of PGE₂ biosynthesis and signaling prior to transplant, recovering CXCR4 receptor expression on mobilized HSC and HPC and significantly increasing repopulation in lethally irradiated recipients. While this staggered dosing regimen is easy to implement, and can be rapidly translated to the clinic, future studies should also investigate short-term pulse of

an NSAID facilitated graft *ex vivo* with PGE₂ immediately prior to transplantation to restore PGE₂ positive tone on CXCR4/homing as we have described (Hoggatt et al., 2009), thus possibly eliminating the need for staggered dosing of NSAID and mobilizing agent.

We have explored several possible mechanisms responsible for the enhanced mobilization resulting from NSAID treatment. While it is tempting to speculate that NSAID facilitated mobilization is a result of the aforementioned reduction in CXCR4, this does not completely explain the effect, since NSAIDs are highly synergistic with the CXCR4 antagonist AMD3100, and the requirement for multiple days of NSAID administration to observe mobilization effects does not correspond to the kinetics of CXCR4 receptor down-regulation. We have clearly demonstrated that NSAIDs increase the HPC pool within the bone marrow, and this pool can be synergistically mobilized with both G-CSF and AMD3100. This suggests that the increases in mobilization may be the result of an increase in “available HPCs” for mobilization. Put simply, if there are more HPCs in the bone marrow, there are more that can potentially leave the bone marrow. However, while we see an increase in total HPC within the bone marrow, data thus far does not indicate expansion of more primitive HSC, yet our phenotypic and transplant studies clearly indicate elevated levels of HSC in the mobilized graft. However, it should also be pointed out that we do not see a reduction in HSC, clearly indicating that HPC expansion does not occur at the

expense of HSC self-renewal, perhaps indicative of an asymmetric self-renewal fate decision.

Osteoblasts within the endosteal bone marrow niche are a significant regulatory component of hematopoiesis (Arai et al., 2004; Calvi et al., 2003; Visnjic et al., 2004; Zhang et al., 2003). Activation of the parathyroid hormone (PTH) receptor in osteoblasts expands HSC by ~4-fold (Taichman et al., 2000; Taichman and Emerson, 1994), and co-transplantation of osteoblasts along with HSC increases engraftment (El Badri et al., 1998). Effects of NSAIDs on osteoblasts have been reported (Chang et al., 2005; Kellinsalmi et al., 2007; Li et al., 2010), and our histological findings in the femurs of NSAID treated mice clearly show reductions in the endosteal osteoblast layer. These findings suggest that the enhanced mobilization of SLAM SKL and LTRCs we observed may result, at least in part, from loss of endosteal niche support and tethering, allowing for release of HSC from the niche and egress into the peripheral blood. While these findings are exciting, it should be noted that most reports on NSAID reductions in osteoblast numbers used NSAID exposures considerably longer than our 4 day regimen. Stimulation of osteoblasts and bone formation by PGE₂ has been attributed to EP4 receptor signaling and antagonism of the EP4 receptor and its downstream signaling results in a reduction in osteoblasts and promotes osteoclast bone resorption (Li et al., 2010; Shamir et al., 2004; Tomita et al., 2002). We found that enhanced mobilization by NSAIDs is the result of a reduction in EP4 signaling, and EP4 antagonists are able to mimic the effects of

NSAID to at least the same degree. These results are highly suggestive that disruption of the osteoblastic endosteal niche within the bone marrow microenvironment by NSAIDs, and specifically EP4 antagonism, may mediate the enhancement in mobilization and should be further explored. Coincidentally, we have demonstrated *in vitro* that EP4 antagonism blocks the inhibition of myelopoiesis by PGE₂. This suggests a unifying mode of action of PGE₂ on hematopoiesis mediated through the EP4 receptor. NSAID enhanced mobilization results from reduced PGE₂ signaling through the EP4 receptor, allowing HPC expansion and reduction of the HSC niche support in the bone marrow, culminating in increased HSC and HPC mobilization. Specifically antagonizing EP4, while retaining normal PGE₂ signaling via EP1-3, is a highly attractive pharmaceutical target, since signaling through EP1-3 protects the stomach and intestine (Kunikata et al., 2001; Takeuchi et al., 2003). Thus, specific antagonism of EP4 may induce the HPC expansion and mobilization effects while retaining the positive GI protective effects of PGE₂ (Houchen et al., 2003), particularly in transplantation settings and cases of hematopoietic injury, which is discussed further in Chapter 4.

As has been discussed throughout this dissertation, it is clear that within the eicosanoid system there is a dynamic balance between signaling of prostaglandins, leukotrienes, and cannabinoids, and that this balance can be shifted to alter homeostasis and generate physiological effects. Intriguingly, in numerous systems, some of which have been reviewed in Table 1, cannabinoids

act in an opposing fashion to PGE₂, possibly acting as endogenous regulators of each other. We would expect then that many of the effects attributed to PGE₂ could be mimicked by cannabinoid antagonists, or that many of the effects attributed to cannabinoids could be mimicked by PGE₂ antagonism. We have shown that a reduction in PGE₂ signaling, either through NSAID administration or EP4 antagonism, enhances mobilization and cannabinoid agonism elicits a similar response. It is tempting to speculate that combination therapies employing both strategies would further enhance hematopoietic mobilization. Further studies evaluating this “yin and yang” relationship are likely to lead to novel pharmaceutical approaches. In particular, in light of our previous findings on the ability of PGE₂ to increase homing, and our recent findings of down-regulation of CXCR4 and VLA-4 by cannabinoids, it is tempting to speculate that cannabinoid antagonism, with or without additional PGE₂ agonism, may further enhance homing/engraftment of HSC to the bone marrow. Studies are ongoing in our laboratory to explore these possibilities.

Chapter 4. Recovery From Hematopoietic Injury by Modulating PGE₂ Signaling or HIF-1 α Post-Irradiation

Introduction:

With the proliferation of nuclear weapons, increasing use of nuclear power, and the advent of worldwide radical terrorism, there is an increasing need and research emphasis on developing countermeasures in the event of a radiological mass casualty event (Moulder, 2004; Pellmar and Rockwell, 2005; Poston, Sr., 2005). As discussed in Chapter 1, HSC and HPC are constantly proliferating to supply the upwards of a trillion cells a day (Ogawa, 1993) needed to maintain homeostasis. This constant state of proliferation make HSC and HPC highly radiosensitive (Chinsoo and Glatstein, 1998; Hall, 2000a), meaning that most, if not all, successful countermeasures will need to account for the hematopoietic system. Regulation of hematopoiesis at this radiation sensitive stage is controlled through accessory cell produced cytokines and growth factors and interactions with the microenvironmental niche stromal cells themselves (Broxmeyer and Smith, 2009; Shaheen and Broxmeyer, 2009). It is known that radiation damage to the hematopoietic system occurs, both at the levels of HSC and HPC, and through changes to cells within the marrow microenvironment that provide signals for their self-renewal, proliferation, survival, differentiation, and migration (Broxmeyer et al., 1976; Coleman et al., 2003; Dainiak et al., 2003; MacVittie et al., 2005; Till and McCulloch, 1964), and that substantive damage to

bone marrow causes the hematopoietic syndrome of the acute radiation syndrome (HS-ARS).

HS-ARS is characterized by life-threatening lymphocytopenia, neutropenia, and thrombocytopenia, and possible death due to infection and/or bleeding. Doses <2 Gy do not cause significant bone marrow damage (Anno et al., 1989); however, at doses of 2-8 Gy the acute radiation syndrome develops proportional to radiation dose, resulting in development of cytopenias and marrow failure in ensuing weeks post exposure (Chinsoo and Glatstein, 1998; Hall, 2000a; Wald, 1982), with the resultant sequela of infection, bleeding and deficient wound healing, in the absence of treatment (Coleman et al., 2003; Dainiak et al., 2003). While bone marrow HSC and HPC are susceptible to radiation exposure, surviving populations of these cells can recover hematopoiesis, given critical time to repair DNA damage, self-renew, expand and differentiate.

The unpredictability of a mass casualty radiation event requires development and utilization of post exposure mitigators of radiation injury with appropriate ease of administration, stability for purposes of stockpiling, ability for rapid distribution and a window of efficacy. In addition, faced with the complexities of a mass casualty event and difficulty of individual dosimetry and triage, interventions that can mitigate or reduce the severity of exposure, but that are benign to those individuals with limited or no exposure are required.

In Chapter 2, we reported positive effects on HSC by PGE₂ treatment, both decreasing apoptosis through up-regulation of Survivin, and increasing self-renewal division and homing/engraftment in the bone marrow. In addition, results described in Chapter 3 clearly demonstrate that reduction of PGE₂ signaling by inhibiting PGE₂ biosynthesis with NSAID administration results in a rapid expansion of HPC in bone marrow. We hypothesized that similar strategies modulating PGE₂ signaling post-irradiation could both protect HSC from radiation induced apoptosis and/or lead to quicker hematopoietic recovery, resulting in increased survival. In this Chapter, we show that treatment with dmPGE₂ shortly following irradiation or delayed administration of Meloxicam post-irradiation results in significantly enhanced hematopoietic recovery and survival. In addition, since many radioprotecting agents, including PGE₂, increase hypoxia (Allalunis-Turner et al., 1989; Glover et al., 1984; Purdie et al., 1983), we explored the potential of HIF-1 α modulation for radiomitigation and report increases in hematopoietic recovery and survival.

Materials and Methods:

Mice

Male and female C57Bl/6 mice were purchased from Jackson Laboratories at 10-12 weeks of age. Mice were housed in microisolator cages (5 mice per cage) with sterilized direct contact bedding (Alpha Dri). Animal holding rooms were maintained at 21 \pm 3 °C with 30 to 80% relative humidity, with at

least 10 air changes per hour of 100% fresh air, and a 12 hour light/dark cycle.

Mice were fed *ad libitum* with commercial rodent chow (Harlan 2018SXC) in cage hoppers and acidified (pH 2.0 - 3.0) water in sipper tube bottles. The Institutional Animal Care and Use Committee of IUSM approved all protocols.

Radiation

Mice were placed in single chambers of a plexiglass irradiation pie (Braintree), with 15 mice per pie, alternating groups of males and females within the same pie. Each group of mice irradiated together in the same pie were divided equally among all treatment groups to ensure that each group received the same irradiation exposure conditions. Mice were irradiated between 9:00 a.m. and 11:00 a.m. from a ¹³⁷Cesium gamma radiation source (GammaCell 40; Nordion International, Kanata, Ontario, Canada) at an exposure rate ~63 cGy per minute, and received 796 cGys total exposure.

Post-irradiation treatment

Irradiated mice were identified by ear punches and treated with either a single subcutaneous dose of dmPGE₂ (40 µg/mouse) or vehicle control at 6 hours post irradiation (N=20 mice per group, evenly split male/female); 6 mg/kg Meloxicam dosed once daily on days 2 through 5 post-irradiation or vehicle control (N=20 or 40 mice per group, respectively, evenly split male/female); or a single subcutaneous dose of CoCl₂ (60 mg/kg) at 6 hours post irradiation (N=20

mice per group, evenly split male/female). One mouse per treatment group were housed in each individual cage.

Morbidity and mortality monitoring

Mice were observed for morbidity or mortality once daily during the acclimation period and twice daily starting on the day after irradiation for thirty days. Moribund mice were scored for signs of early euthanasia based on three parameters: the severity of hunched posture, squinted/closed eyes, and decreased activity. Each criteria was scored on a scale of 1 to 3, with 3 being the most severe. Moribund mice with a score of 8 or 9 were euthanized and the date of death was recorded.

Colony assays

After 35 days, remaining irradiated mice were sacrificed, bone marrow acquired from femurs, and total CFC including CFU-GM, BFU-E and CFU-GEMM were enumerated in 1% methylcellulose/IMDM containing 30% HI-FBS, 1 U/ml rhEPO, 10 ng/ml rhGM-CSF and 50 ng/ml rmSCF as described (Broxmeyer et al., 2007a; Fukuda et al., 2007). All cultures were established in triplicate from individual animals, incubated at 37 °C, 5% CO₂, 5% O₂ in air for 7 days and colonies quantitated by microscopy.

Results:

PGE₂ treatment increases survival post-irradiation

PGE₂ biosynthesis is increased following γ -radiation and can result from up-regulation of cPLA₂ (Chen et al., 1996) or COX2 (Isoherranen et al., 1999). In rats, spinal cord irradiation elevates PGE₂ levels within 3-24 hours that persist for 3 days (Siegal and Pfeffer, 1995). In mice, brain irradiation induces COX-dependent PGE₂ production and elevated levels of PGE synthases (Moore et al., 2005). In breast cancer patients, radiation therapy triggers monocyte PGE₂ production (Cayeux et al., 1993) and in leukemia and lymphoma patients undergoing autologous transplant, plasma PGE₂ levels were 3-12 fold higher than controls between days 0 and 10 post-transplant (Cayeux et al., 1993). High PGE₂ levels occurred when patients were cytopenic, suggesting that PGE₂ was produced by cells less sensitive to cytoreductive therapies. Due to the anti-apoptotic and self-renewal properties of PGE₂ signaling, as described in Chapter 2, it is possible that up-regulation of PGE₂ synthesis is an endogenous mechanism for radioprotection. Although PGE₂ is endogenously produced as a consequence of radiation damage, exogenous administration, particularly using the metabolically stable dmPGE₂ analog is likely to be more efficacious and maintain higher levels of active PGE₂ to positively affect HSC survival and function. Early studies have explored the use of dmPGE₂ administered prior to radiation exposure (Hanson, 1987; Hanson and Ainsworth, 1985; Walden, Jr. et al., 1987; Walden, Jr. and Farzaneh, 1995); however, in the case of a mass

casualty event, prophylactic administration is not feasible, and little research has explored the use of dmPGE₂ post-irradiation as a “radiomitigator” rather than a “radioprotector”.

We performed a preliminary test of hypothesis for mitigating the damage to hematopoietic cells post irradiation with dmPGE₂ treatment, using a murine HS-ARS model developed by Dr. Orschell at IUSM. Irradiated mice were treated with a single subcutaneous dose of dmPGE₂ or vehicle control at 6 hours post irradiation and moribund status and mortality were monitored for 30 days post-irradiation. Single dmPGE₂ treatment at 6 hours post-irradiation resulted in 95% survival (P=0.0011) compared to 50% survival in control mice (Figure 38A). In normal, non-irradiated mice, total CFC (CFU-GM + BFU-E + CFU-GEMM) are generally in the range of 40,000 per femur. In control mice that received 796 cGys and survived to day 35, a significant deficit in marrow HPC is evident (Figure 38B). However, in mice treated with dmPGE₂, marrow HPC were still lower than historical controls, but were significantly higher than control irradiated mice (Figure 38B), indicating that hematopoiesis is more robust, likely as a result of enhanced stem cell repair, self-renewal and HSC and HPC expansion. These results indicate that dmPGE₂ is a highly effective radiomitigator.

Figure 38

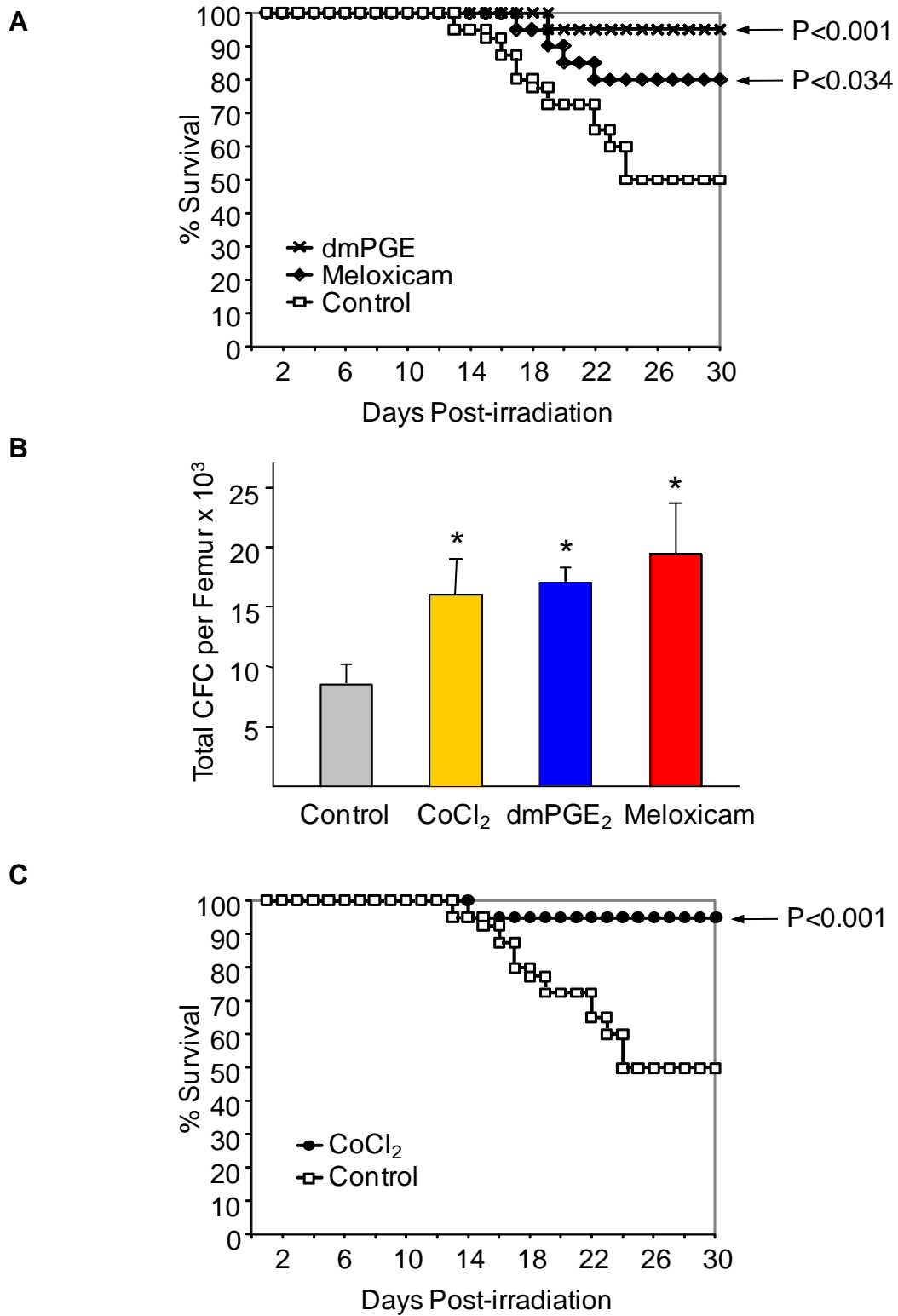


Figure 38. Radiomitigation by treatment with dmPGE₂, Meloxicam or CoCl₂.

(A) Cohorts of 20-40 mice were irradiated with 796 cGys from a ¹³⁷Cs source.

Mice received a single dose of dmPGE₂ (40 µg/mouse) at 6 hours post irradiation, or 6 mg/kg Meloxicam dosed daily on days 2-5 post irradiation.

Animals were monitored for morbidity and mortality twice/daily for 30 days and euthanized when moribund. Survival curves were analyzed with a log-rank test.

(B) Analysis of total CFC (CFU-GM, BFU-E and CFU-GEMM) in methylcellulose culture. Data are Mean ± SEM from N=5 mice per group, each assayed individually.

(C) Cohorts of 20 mice were irradiated with 796 cGys from a ¹³⁷Cs source. Mice received a single dose of CoCl₂ (60 mg/kg) at 6 hours post irradiation. Animals were monitored for morbidity and mortality twice/daily for 30 days and euthanized when moribund. Survival curves were analyzed with a log-rank test.

Radiomitigation with delayed NSAID administration

While it is clear that exposure to PGE₂ early post-irradiation increases survival, continued exposure to PGE₂ is inhibitory to HPC expansion (Gentile and Pelus, 1988; Kurland et al., 1978; Kurland et al., 1979; Pelus et al., 1979; Pelus et al., 1981; Pelus et al., 1983; Pelus et al., 1988; Pelus and Gentile, 1988). By reducing PGE₂ biosynthesis with a delayed administration of an NSAID, HPC inhibitory signaling by PGE₂ would be ablated, and rapid hematopoietic expansion could occur, allowing for repopulation of the irradiated animal. Work discussed in Chapter 3 has demonstrated that Meloxicam administration, in non-irradiated mice, can increase HPCs within the bone marrow, leading to an increase in mature blood cells. To assess if Meloxicam administration post-irradiation would lead to increased survival, irradiated mice were treated with 6 mg/kg Meloxicam dosed once daily on days 2 through 5 post irradiation and moribund status and mortality were monitored for 30. This delayed regimen of Meloxicam post-irradiation resulted in 80% survival (P=0.034) compared to 50% survival in control mice (Figure 38A). Similarly to our analysis of dmPGE₂ treatment, we also evaluated total CFC content in femurs 35 days post-irradiation, as a measure of hematopoietic recovery. Meloxicam administration significantly increased bone marrow CFC content compared to control, indicating that delayed NSAID administration post-irradiation can expand the hematopoietic compartment and increase survival in treated animals.

Cobalt chloride administration increases survival post-irradiation

PGE₂ is a known transcriptional inducer of HIF-1 α (Fukuda et al., 2003; Jung et al., 2003; Kaidi et al., 2006) and stabilizes HIF-1 α protein (Liu et al., 2002; Piccoli et al., 2007). HIF-1 α is a key transcriptional regulator with a broad repertoire of downstream target genes and is responsible for physiological adaptation from normoxia (21% O₂) to hypoxia (1% O₂) (reviewed in (Ke and Costa, 2006)). HIF-1 α up-regulates EPO production (Semenza et al., 1991), the anti-apoptotic protein Survivin (Peng et al., 2006; Wei et al., 2006; Yang et al., 2004), numerous cell proliferation and survival genes (Cormier-Regard et al., 1998; Feldser et al., 1999; Krishnamachary et al., 2003), the angiogenic growth factor VEGF (Levy et al., 1995) and others. The HSC bone marrow niche is hypoxic (Levesque et al., 2007), and it has been suggested that this hypoxic niche maintains HIF-1 α activity that maintains stem cells (Lin et al., 2006). Hypoxic conditions expand human HSC (Danet et al., 2003) and HPC (Broxmeyer et al., 1989; Broxmeyer et al., 1990; Smith and Broxmeyer, 1986) *in vitro*, creating a role for HIF-1 α in HSC maintenance. In addition, HIF-1 α has recently been reported to prevent hematopoietic cell damage caused by overproduction of reactive oxygen species (ROS) (Kirito et al., 2009). While the damaging effects of radiation exposure have largely been attributed to direct DNA damage (Hall, 2000b), it is now well recognized that the radiation damaging effects on HSC are also mediated by other stress response pathways including oxidative stress. ROS have been implicated in mediating chronic oxidative stress

resulting from radiation-induced late morbidity in long-term cancer survivors (Zhao et al., 2007). Oxidative stress-mediated radiation injury of hematopoietic (Nunia et al., 2007) and non-hematopoietic cells (Ishii et al., 2007; Wan et al., 2006), and the use of free radical scavengers to reverse the damage, has been previously documented (Ishii et al., 2007; Rabbani et al., 2005; Sandhya et al., 2006; Wan et al., 2006). The reduction of ROS induced-damage, coupled with the up-regulation of erythropoiesis, angiogenesis, cell survival/proliferation, DNA repair and anti-apoptotic functions of HIF-1 α support a hypothesis of altering HIF-1 α as a radiomitigation strategy.

Numerous radioprotectors, including dmPGE₂, cysteamine, 5-HT, and the FDA approved compound Amifostine, induce marrow hypoxia, and only doses of the compounds that induce sufficient hypoxia are radioprotective (Allalunis-Turner et al., 1989; Glover et al., 1984; Purdie et al., 1983). While the effects of Amifostine are traditionally thought to be due to free radical scavenging, it has been suggested that this induction of marrow hypoxia may play an important role for radioprotection (Kouvaris et al., 2007), further indicating that compounds which induce HIF-1 α may be potent radioprotectors. Cobalt chloride (CoCl₂) is a known potent inducer and stabilizer of HIF-1 α (Ke et al., 2005; Salnikow et al., 2004; Wang and Semenza, 1993; Yuan et al., 2003), and even before its mechanism of action was known, CoCl₂ was used to treat anemia in pregnant women, infants, and hemodialysis patients (Holly, 1955).

In one case, CoCl_2 was explored immediately after irradiation and was found to have positive effects on erythropoietic recovery and survival (Vittorio and Whitfield, 1971), yet its use as a radiomitigator is largely unexplored. In parallel with our previously described studies exploring the role of altering PGE_2 signaling for radiomitigation, irradiated mice were treated with a single subcutaneous dose of CoCl_2 (60 mg/kg) at 6 hours post-irradiation and moribund status and mortality were monitored as described. Single CoCl_2 treatment at 6 hours post-irradiation resulted in 95% survival ($P=0.0011$) (Figure 38C), similar to the effectiveness of dmPGE_2 . In addition, as was the case with dmPGE_2 and Meloxicam, increased survival in CoCl_2 treated mice correlated with significantly increased CFC in bone marrow (Figure 38B), indicating that CoCl_2 increased hematopoietic recovery and expansion post-irradiation.

Discussion:

These studies outline three different pharmaceutical strategies for radiomitigation. In the context of a radiation incident, particularly one in a densely populated area where there are numerous affected (and non-affected) individuals, strategies that can be employed quickly and safely to the masses are ideal. While hematopoietic transplantation may be able to treat patients after exposure to radiation, the logistics and timing involved in a mass casualty situation make transplantation impractical. Therefore, the ideal treatment should be able to be stockpiled and distributed to a large amount of people after an incident. Currently, the Centers for Disease Control and Prevention (CDC) have

several compounds in the Strategic National Stockpile (SNS) in the case of a radiological incident. These include the decorporation agents Prussian Blue (ferrihexacyanoferrate (II)) and Diethylenetriaminedipentaacetate (DTPA), which work by binding to radioactive isotopes in the body and preventing entrance into the bloodstream and facilitating excretion (Kargacin and Kostial, 1985), and Neupogen[®] (G-CSF), for expansion of hematopoiesis to treat HS-ARS. While these agents are relatively safe and can easily be stockpiled, Prussian Blue and DTPA only serve to help reduce radioisotopes in the body, and do not treat already existing consequences of radiation exposure. Treatment with Neupogen[®] and other recombinant growth factors have shown some modest success for radiomitigation (Herodin et al., 2003; Herodin and Drouet, 2005; MacVittie et al., 2005); however, these compounds require administration through injections, and given the known occurrences of adverse events associated with these compounds, they are less attractive for distribution to a large population, since many of the individuals seeking care after a radiation event are likely to have received little to no exposure warranting treatment. Taking into consideration these issues, Meloxicam is a highly attractive radiomitigation agent: it is safe to distribute to a broad spectrum of patients, both those exposed and non-exposed to radiation, it has relatively few side effects, and it can be easily added to the SNS and is essentially already stockpiled in micro-distribution centers, since most pharmacies currently carry it in stock.

Work by others has explored NSAID administration prior to irradiation in mice to increase hematopoietic recovery (Kozubik et al., 1994; Nishiguchi et al., 1990; Pospisil et al., 1989; Serushago et al., 1987). Two recent studies exploring the use of Meloxicam post-irradiation report contradictory results. One study by Hofer et al. exploring hematologic parameters in mice after sub-lethal irradiation followed by 4 days of Meloxicam showed increased hematopoietic recovery (Hofer et al., 2006), while another study by Jiao et al. showed a marginal decrease in survival with 7 days administration of Meloxicam, and greater decreases in survival using the highly COX2 selective drug Celecoxib (Jiao et al., 2009) in post-irradiated mice. Our results clearly demonstrate enhanced survival and hematopoietic recovery in mice treated with 796 cGys when Meloxicam was administered daily post-irradiation for 4 days, starting on Day 2 (Figure 38A). It is important to highlight that the study showing a detrimental effect of NSAID administered after radiation exposure began NSAID administration immediately following radiation exposure, while the studies performed by us or by Hofer et al. delayed administration of NSAID for 1 or 2 days, respectively. Furthermore, it is important to note that our results also clearly indicate that early exposure to PGE₂ facilitates hematopoietic recovery and survival in irradiated mice (Figure 38A). In light of this data, we have formed a “just right (Goldilocks)” hypothesis for PGE₂ exposure and post-irradiation survival. Too little PGE₂ in the early period post-irradiation reduces positive anti-apoptotic and self-renewal effects, while too much PGE₂ signaling at later time points inhibits HPC expansion and

reduces hematopoietic recovery. In contrast, early exposure to PGE₂ to reduce apoptosis and stimulate self-renewal, followed later by exposure to an NSAID to block PGE₂ inhibition of HPC expansion produces an environment that fosters reconstitution of hematopoiesis. Based on our model, we would predict the detrimental effects observed by the early administration of NSAID by Jiao et al. (Jiao et al., 2009), since it would block the positive effects of endogenously produced PGE₂ on HSC survival (Cayeux et al., 1993; Chen et al., 1996; Isoherranen et al., 1999; Moore et al., 2005; Siegal and Pfeffer, 1995) leading to reduced hematopoietic recovery and increased mortality in these treated mice. However, our strategy of allowing/utilizing PGE₂ to exert positive effects early post-irradiation, then blocking its myelosuppressive effect later with Meloxicam, would predict facilitation of hematopoietic expansion and recovery, as we demonstrated.

Our recent findings on the effects of NSAID administration on the endosteal osteoblastic niche (Figure 35) in non-irradiated mice may indicate that administration of NSAIDs too early would have detrimental effects on the already compromised HSC microenvironment, reducing the available niche support to reconstitute hematopoiesis. As has been thoroughly discussed, PGE₂ is a known anabolic stimulator of bone formation. It has recently been demonstrated that the antiapoptotic protein Survivin is necessary for the survival of megakaryocyte progenitors (Wen et al., 2009). As we showed in Chapter 2, PGE₂ up-regulates Survivin transcription and expression in hematopoietic cells.

Moreover, an important role of megakaryocytes in supporting osteoblasts after irradiation has been demonstrated (Dominici et al., 2009). It is therefore possible that PGE₂ treatment enhances megakaryocyte Survivin expression post-irradiation, increasing the total number of megakaryocytes available to enhance the hematopoietic niche support. To further explore these mechanisms, in collaboration with Dr. Kacena at IUSM, femurs from irradiated mice treated with dmPGE₂ or control were examined. Preliminary results indicate a substantial increase in megakaryocyte numbers in dmPGE₂ treated mice compared to control mice (data not shown), suggesting another possible mechanism for increased survival in these mice, but also suggesting a possible mechanism for the adverse effects seen by Jiao et al. with early administration of NSAID. Further analysis is currently ongoing to assess the role of megakaryocytes/osteoblasts and HSC niche in our radiomitigation strategies.

While we have clearly demonstrated the utility of the eicosanoid pathway, particularly the prostaglandin pathway, in recovery of hematopoiesis after severe radiation injury, the true potential of this pathway for modulating recovery after a wide spectrum of hematopoietic injuries, e.g., chemotherapy, radiotherapy, post-hematopoietic transplant, remains underexplored. Further mechanistic based studies are ongoing to define the therapeutic utility of individual members of this pathway and combination approaches to develop additional strategies for recovery from hematopoietic injury.

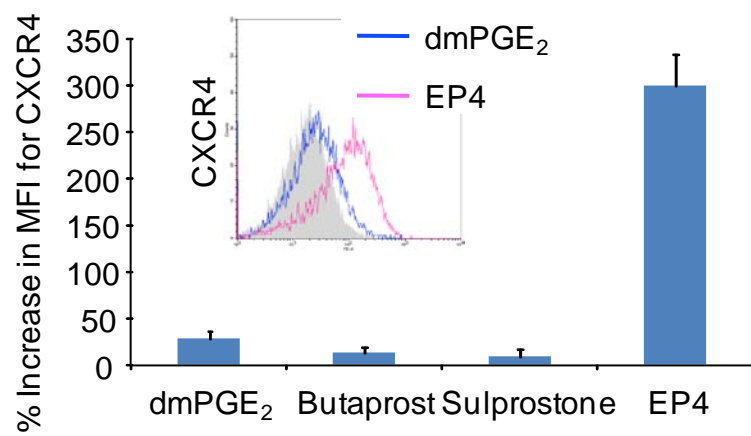
Chapter 5. Future Directions

***Ex vivo* pulse with PGE₂:**

We have provided clear evidence that a short, *ex vivo* pulse of a hematopoietic graft prior to transplantation increases HSC and HPC CXCR4 receptor expression and homing/engraftment in the bone marrow (Hoggatt et al., 2009). However, the exact mechanisms behind this enhancement in CXCR4 receptor expression and homing are still largely unknown. In particular, a pulse with dmPGE₂ is presumably activating multiple different EP receptors on HSC, and since the ultimate signaling within the cell is the culmination of the signaling events of all the receptors, it is possible that a focused agonism of only the EP receptor(s) involved in the increase in homing would lead to a substantially greater therapeutic product. Early work in our laboratory has begun to explore which EP receptor is responsible for enhancing homing by examining CXCR4 receptor expression after *in vitro* treatment with EP receptor specific agonists and antagonists. Exciting preliminary studies indicate that specific agonism of the EP4 receptor with L-902,688 increases CXCR4 receptor expression 299±34% compared to 29.8±9.1% for dmPGE₂, while the EP2 agonist Butaprost and EP1/3 agonist Sulprostone had little effect (Figure 39A). In addition, this increase in CXCR4 in response to the specific EP4 agonist could be attenuated by pre-treatment with the EP4 antagonist L-161,982, but was not affected by the EP1-3 antagonist AH6809 (Figure 39B). These results were only seen in human CD34⁺

Figure 39

A



B

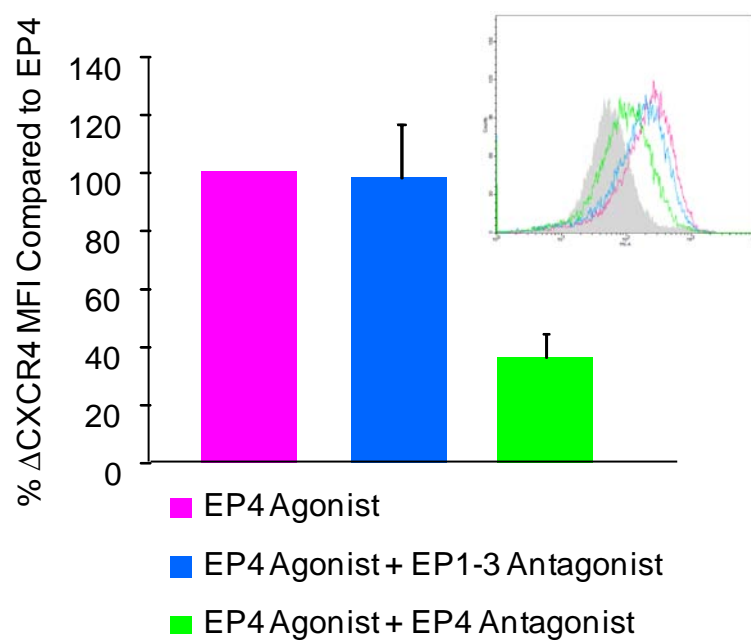


Figure 39. Specific agonism of EP4 increases CXCR4 receptor expression on CD34⁺ UCB cells.

(A) MACS[®] selected CD34⁺ cells from UCB samples were pulsed with dmPGE₂, the EP2 agonist Butaprost, the EP1/3 agonist Sulprostone, or the EP4 agonist L-902,688. MFI of expression of CXCR4 on CD34⁺ cells was determined.

(B) MACS[®] selected CD34⁺ cells from UCB samples were pulsed with the EP4 agonist L-902,688 with or without a 30 minute pre-incubation with the EP1-3 antagonist AH6809, or the EP4 antagonist L-161,982. MFI of expression of CXCR4 on CD34⁺ cells was determined.

cells, and were not seen with mouse SKL cells, likely indicating either species specific differences in the binding and signaling properties of the EP4 pharmaceutical, species differences in downstream signaling events that lead to CXCR4 up-regulation, or differences in kinetics of response or culture conditions. In either case, future studies should focus on EP specific agonism to increase CXCR4 and homing, particularly if the large increases in CXCR4 seen with EP4 agonism are recapitulated with similar magnitude in *in vivo* homing studies.

Additional agents to increase homing:

It is interesting to note that Survivin (Peng et al., 2006; Wei et al., 2006; Yang et al., 2004) and CXCR4 (Phillips et al., 2005; Staller et al., 2003; Wang and Semenza, 1993) transcription, which we have shown to be increased by PGE₂, are both up-regulated by the transcription factor HIF-1 α , which can be stabilized (Liu et al., 2002; Piccoli et al., 2007) and transcriptionally increased (Fukuda et al., 2003; Jung et al., 2003; Kaidi et al., 2006) by PGE₂, potentially linking these PGE₂ responsive pathways. In addition, HIF-1 α has been demonstrated to increase HPC homing to gliomas (Tabatabai et al., 2006), and generally regulates genes associated with migration (Ricciardi et al., 2008). Future studies should explore the role of HIF-1 α in HSC and HPC homing and engraftment and should utilize agents like CoCl₂, perhaps in combination with PGE₂, Diprotin A (Christopherson et al., 2004), or other agents known to increase homing, to develop novel homing therapeutic strategies. In light of our findings that cannabinoid agonism decreases CXCR4 and facilitates mobilization,

cannabinoid antagonists, many of which function as inverse agonists (Pertwee, 2005), are potential agents that can be used to facilitate homing of HSC and HPC. In addition, the use of multiple agents in combination may lead to synergistic increases in mobilization. Inhibition of CD26 has been shown to increase HSC and HPC homing to the marrow (Christopherson et al., 2004), and the CD26 inhibitor Sitagliptin is currently being explored clinically to improve UCB hematopoietic transplantation. Eicosanoid based strategies in combination with CD26 inhibition should be explored to enhance HSC homing and engraftment.

Mobilization of bone marrow-derived progenitor cells:

Bone marrow derived progenitors have been implicated in neoangiogenesis. Circulating endothelial progenitor cells (EPC) are found at low levels in mouse bone marrow and peripheral blood (Aicher et al., 2005; Orlic et al., 2001a; Orlic et al., 2001b) and in the CD34⁺ adult blood and cord blood populations (Asahara et al., 1997; Rafii and Lyden, 2003), and can be mobilized by ischemic injury, cytokines such as GM-CSF and G-CSF, SDF-1 α and vascular endothelial growth factor (VEGF) (Aicher et al., 2005; Asahara et al., 1999; Hattori et al., 2001; Orlic et al., 2001a; Powell et al., 2005; Takahashi et al., 1999; Wolfram et al., 2007). Mobilization of EPC represents a possible novel therapeutic option to enhance neovascularization. In animal models, mouse and human EPCs have been shown to partially rescue cardiovascular dysfunction following ischemic hind limb or myocardial injury, with evidence for contribution to new vessel growth and re-endothelialization (Dimmeler et al., 2005; Hu et al.,

2006; Orlic et al., 2001a; Rafii and Lyden, 2003). Clinically, intracoronary EPC administration has resulted in enhanced neovascularization with beneficial post-infarct remodeling (Assmus et al., 2002; Strauer et al., 2002), but with little evidence for EPC engraftment in newly formed blood vessels (Dimmeler et al., 2005; Hristov and Weber, 2006). Since most EPC populations are defined by biomarkers that are shared by hematopoietic cells and also contain CD14⁺ monocytic cells, it is likely that reported positive effects result from paracrine effects of HSC and HPC driving the angiogenic process (Kopp et al., 2006; Willett et al., 2005).

The fact that potent mobilizers such as G-CSF also exert pro-inflammatory capacity that may enhance atherosclerosis in patients with coronary artery disease (Aicher et al., 2005), suggests the need for additional methods to enhance the frequency of EPC. The fact that we observe a significant increase in CD34⁺ cells in baboons and HSC and HPC in mice treated with the NSAID Meloxicam strongly suggests the possibility of NSAID-mediated mobilization of EPC populations. In fact, a recent study showed that low dose aspirin promotes EPC migration and prevents senescence (Hu et al., 2008). It also is tempting to hypothesize that mobilization of EPC or circulating angiogenic cells may contribute to the well known effects of aspirin in preventing major adverse cardiac events and mortality (Freimark et al., 2002; Leon et al., 1998) in addition and in coordination with aspirin's already known anti-platelet activity. It is interesting to note that the highly COX2 selective NSAID Valdecoxib was recently withdrawn

from the market due to adverse cardiovascular events, and our studies demonstrate that Valdecoxib is unable to mobilize HSC and HPC from bone marrow, while Aspirin is highly effective, possibly explaining some of the cardiovascular differences between these two compounds.

A critical issue currently restricting the field is a lack of unique biomarkers to define EPC (Yoder, 2009) and it is imperative to define EPC at a clonogenic level. We have recently received IRB approval to study the ability of a 5 day regimen of Meloxicam to mobilize CD34⁺ cells as well as EPCs. Our collaborator, Dr. Yoder and his colleagues have recently utilized a functional approach to define a rare population of circulating endothelial colony forming cells (ECFC) in adult human peripheral blood that appear to be true EPC (Ingram et al., 2004; Yoder et al., 2007). ECFC express markers of primary endothelium (CD31, 105, 144, 146, VWF, KDR and UEA1) but lack hematopoietic cell markers. They clonally propagate with high proliferative capacity and replate into secondary and tertiary ECFC, and form capillary structures *in vitro*, and human blood vessels *in vivo* in immunodeficient mice with incorporation into the murine vasculature (Yoder et al., 2007). We have recently obtained IRB approval to conduct a healthy volunteer study to evaluate the potential for Meloxicam to mobilize ECFCs, and if so, future studies will determine what contribution this mobilization has to neovascularization and cardiac repair.

Mobilization with other agents:

Similar to the future studies previously proposed for EP receptor specific effects on HSC homing, receptor analysis studies should continue to progress for hematopoietic mobilization. The results presented in Chapter 3 suggest that antagonism of the EP4 receptor will facilitate HPC expansion and reduction of the HSC niche support in the bone marrow, culminating in increased HSC and HPC mobilization. Specifically antagonizing the EP4 receptor, while retaining normal PGE₂ signaling via EP1-3, is a highly attractive pharmaceutical target, since signaling through EP1-3 protects the stomach and intestine (Kunikata et al., 2001; Takeuchi et al., 2003), which may allow for increased dose escalation for greater mobilization responses. In addition, EP4 antagonists are already being explored in clinical trials (Maubach et al., 2009), suggesting that rapid translation to the clinic may be possible for EP4 antagonist based mobilization strategies.

The incidence of chronic GVHD is higher (Couban et al., 2002; Cutler et al., 2001; Mohty et al., 2002) for G-CSF-mobilized PBSC than bone marrow. In contrast, GVHD is significantly lower in patients receiving GM-CSF mobilized PBSC grafts compared to G-CSF; however mobilized CD34⁺ counts are significantly lower (Devine et al., 2005). We have demonstrated that NSAID co-administration significantly increases mobilization of HSC and HPC, including CD34⁺ cells in baboons, independent of mobilizing mechanisms of the co-administered mobilizer. These results suggest that NSAIDs can increase CD34⁺

cell mobilization in combination with GM-CSF, overcoming one of the major hurdles for its clinical use, and allowing for robust mobilized hematopoietic grafts with reduced GVHD potential. Similarly, further analysis on the ability of NSAIDs to enhance mobilization of AMD3100, or the combination of AMD3100 and G-CSF (Fukuda et al., 2007; King et al., 2001; Pelus et al., 2006a), should be performed to identify possible mobilizing regimens which are independent of recombinant growth factor administration.

Eicosanoid regulation to improve gene therapy:

Since a single HSC can give lifelong production of all blood lineages, transplantation of HSCs that have been transduced with a gene is a potential curative therapy for many inherited and acquired diseases. However, gene therapies have been hampered due to less than adequate culture conditions used to transduce adult HSC *ex vivo* and their propensity towards apoptosis (Santoni de Sio and Naldini, 2008), and to modification of HSC that alters their function, particularly homing to the marrow microenvironment (Hall et al., 2006). Preculture of HSC prior to viral transduction is common in transduction protocols, but can lead to reduced stemness, (i.e., differentiation and loss of self-renewal) and as a consequence, less long-term engraftment (Dorrell et al., 2000; Gothot et al., 1998; Rebel et al., 1999). *Ex vivo* expansion using hematopoietic growth factor cocktails has also been attempted to increase the number of HSC following gene transduction protocols to speed engraftment and hematopoietic reconstitution, with limited success (Liu et al., 2008; Piacibello et al., 2002; Tesio

et al., 2008). However, it is generally held that there is a loss of long-term repopulating ability, likely due to inadequate culture conditions, introduction of defects that promote apoptosis (Domen et al., 2000; Liu et al., 2003; Wang et al., 2000), disruption of marrow homing efficiency (Orschell-Traycoff et al., 2000; Ramirez et al., 2001; Zhai et al., 2004) and initiation of cell cycle (Giet et al., 2001; Glimm et al., 2000). Therefore, there is considerable need for novel techniques to improve the efficiency and efficacy of gene therapy protocols.

Our data demonstrating that dmPGE₂ pulse exposure enhances self-renewal division and reduces apoptosis in HSC (Hoggatt et al., 2009) form the rationale for a strategy to treat cells with dmPGE₂ prior and/or during the transduction protocol to reduce apoptosis and maintain stemness/initiate symmetrical self-renewal HSC cell division, and enhance homing and engraftment. In addition, a recent report has demonstrated that antagonism of the leukotriene LTB₄ receptor expands CD34⁺ cells in culture (Chung et al., 2005), in agreement with other eicosanoid “yin and yang” hematopoietic effects attributed to leukotrienes (Table 1). We have recently confirmed that treatment of UCB CD34⁺ cells with an LTB₄ antagonist maintains and expands the CD34⁺ population in *in vitro* culture in the presence of growth factors (Figure 40), suggesting that addition of LTB₄ antagonists to viral transduction protocols for gene therapy may improve yield and hematopoietic reconstitution of gene transduced HSC. Further studies should evaluate the use of eicosanoids in current and future gene transduction and gene-transduced engraftment

Figure 40

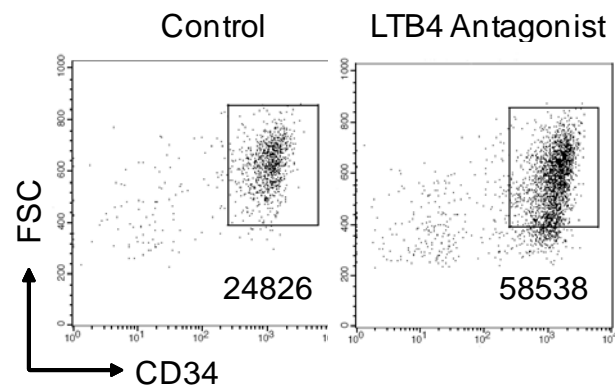


Figure 40. LTB4 antagonism expands CD34⁺ UCB cells.

CD34⁺ cells from UCB samples were expanded in media with 5% HI-FBS, 50 ng/ml SCF, 100 ng/ml TPO and 100 ng/ml Flt-3L for 3 days in the presence of 1 μ M of the LTB4 antagonist U-75302, or vehicle control. CD34⁺ cell were quantitated by FACS. Shown are representative FACS plots with total CD34⁺ cells present in cultures initiated with 5×10^5 CD34⁺ cells.

protocols, particularly considering that the mechanisms of action for enhanced transduction (i.e. reduced apoptosis, increased self-renewal/maintenance of stemness, increased HSC homing, etc.) are likely independent of a particular transduction protocol, allowing for optimization of a broad spectrum of current and future gene therapies.

Clinical trials and patent applications:

Despite its dramatic effect on the field of hematopoietic transplantation, poor mobilization in response to G-CSF occurs in 25% of patients, particularly those with lymphoma and multiple myeloma (Stiff et al., 2000) and 15% of normal donors (Anderlini et al., 1997), requiring extended aphereses (Schmitz et al., 1995). Our data in mice and baboons clearly demonstrates that NSAID co-administration with G-CSF results in a synergistic increase in mobilization, suggesting that the addition of NSAIDs along with G-CSF regimens for autologous mobilization would be clinically beneficial. A phase I/II clinical trial to assess the safety and efficacy of Meloxicam in combination with G-CSF (Filgrastim) for mobilization of autologous PBSC from patients with hematological cancers undergoing autologous PBSC transplantation will soon be conducted at the IUSM Medical Center.

AMD3100 has been used successfully as a single agent to mobilize peripheral blood stem cells (PBSC) from normal donors for allogeneic stem cell transplantation (Devine et al., 2008). Following a single dose of AMD3100 (240

μg/kg) followed by leukapheresis 4 hours later, a median of 2.9 (range, 1.2-6.3) x 10⁶ CD34⁺ cells/kg recipient weight were collected. Furthermore, AMD3100 was safe and well tolerated in normal donors, and mobilized HSPC engrafted successfully (Devine et al., 2008). A potential advantage of AMD3100 mobilized PBSC products is the presence of a greater number of mature T cells (Devine et al., 2008) and plasmacytoid dendritic cell precursors (Rettig et al., 2009) which may improve immune function post-transplantation compared to Filgrastim mobilized stem cells, which have been associated with an increased risk of infections following transplantation (Volpi et al., 2001). In light of these findings, and of our recent findings of the ability of NSAID co-administration to synergistically increase hematopoietic mobilization, we believe that a mobilization strategy utilizing a regimen of NSAID, followed by AMD3100, without any administration of G-CSF, will lead to a superior hematopoietic graft for allogeneic transplantation. A phase I/II clinical trial to assess the safety and efficacy of Meloxicam in combination with AMD3100 for mobilization of PBSC in normal donors for allogeneic PBSC transplantation will be performed at the IUSM Medical Center.

Two separate patent applications have been filed encompassing many of the therapeutic applications discussed throughout this dissertation, including increasing homing through PGE₂ agonism of the EP4 receptor; improving gene therapy with PGE₂ based modalities; and enhancement of hematopoietic mobilization with NSAIDs or EP4 antagonism. These inventions are described in

PCT Patent Application PCT/US09/63654 “Materials and Methods to Enhance Hematopoietic Stem Cell Engraftment Procedures” (Filed 11/06/08), and U.S. Provisional Application 61/261,352 “Inhibition of Prostaglandin E2 (PGE₂) Signaling by Non-Steroidal Anti-Inflammatory Drugs.”

HSC and HPC fate decisions directed by eicosanoids:

Our preliminary findings suggest that PGE₂ facilitates an increase in HSC function, presumably by self-renewal division, and blockade of PGE₂ signaling facilitates HPC expansion. Hematopoietic expansion and maintenance of HSC can occur via symmetrical self-renewal divisions giving two daughter HSCs, symmetrical differentiation divisions giving two daughter cells destined for differentiation, or asymmetric division giving one daughter HSC and one daughter destined to differentiate (reviewed in (Morrison and Kimble, 2006)). Recently, it has been demonstrated that phenotypically defined hematopoietic precursors have the capacity for all three types of division (Wu et al., 2007). Future studies should employ a combination CFC analysis, long-term repopulation assays, and single-cell live tracking (Wu et al., 2007) to determine what effect eicosanoid signaling has on HSC and HPC. For instance, if we see increased progenitor function in a CFC assay, with no difference in long-term repopulating capacity, this would be suggestive of asymmetric division of HSC. If, however, we see a reduction in long-term repopulating capacity, this would be suggestive of symmetric differentiation of HSC.

References

- Abkowitz,J.L., Robinson,A.E., Kale,S., Long,M.W., and Chen,J. (2003). Mobilization of hematopoietic stem cells during homeostasis and after cytokine exposure. *Blood* 102, 1249-1253.
- Adams,G.B., Chabner,K.T., Alley,I.R., Olson,D.P., Szczepiorkowski,Z.M., Poznansky,M.C., Kos,C.H., Pollak,M.R., Brown,E.M., and Scadden,D.T. (2006). Stem cell engraftment at the endosteal niche is specified by the calcium-sensing receptor. *Nature* 439, 599-603.
- Adler,B.K., Salzman,D.E., Carabasi,M.H., Vaughan,W.P., Reddy,V.V., and Prchal,J.T. (2001). Fatal sickle cell crisis after granulocyte colony-stimulating factor administration. *Blood* 97, 3313-3314.
- Adolfsson,J., Borge,O.J., Bryder,D., Theilgaard-Monch,K., Astrand-Grundstrom,I., Sitnicka,E., Sasaki,Y., and Jacobsen,S.E. (2001). Upregulation of Flt3 expression within the bone marrow Lin(-)Sca1(+)c-kit(+) stem cell compartment is accompanied by loss of self-renewal capacity. *Immunity*. 15, 659-669.
- Adolfsson,J., Mansson,R., Buza-Vidas,N., Hultquist,A., Liuba,K., Jensen,C.T., Bryder,D., Yang,L., Borge,O.J., Thoren,L.A., Anderson,K., Sitnicka,E., Sasaki,Y., Sigvardsson,M., and Jacobsen,S.E. (2005). Identification of Flt3+ lympho-myeloid stem cells lacking erythro-megakaryocytic potential a revised road map for adult blood lineage commitment. *Cell* 121, 295-306.
- Aglietta,M., Piacibello,W., and Gavosto,F. (1980). Insensitivity of chronic myeloid leukemia cells to inhibition of growth by prostaglandin E1. *Cancer Res.* 40, 2507-2511.
- Ahmed,M., Khanna,D., and Furst,D.E. (2005). Meloxicam in rheumatoid arthritis. *Expert. Opin. Drug Metab Toxicol.* 1, 739-751.
- Aicher,A., Zeiher,A.M., and Dimmeler,S. (2005). Mobilizing endothelial progenitor cells. *Hypertension* 45, 321-325.
- Aiuti,A., Webb,I.J., Bleul,C., Springer,T., and Gutierrez-Ramos,J.C. (1997). The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. *J. Exp. Med.* 185, 111-120.

- Akashi,K., Traver,D., Miyamoto,T., and Weissman,I.L. (2000). A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* **404**, 193-197.
- Alberich,J.M., Rayman,N., Tas,M., Verbakel,S.E., Battista,N., van,L.K., Lowenberg,B., Maccarrone,M., and Delwel,R. (2004). The peripheral cannabinoid receptor Cb2, frequently expressed on AML blasts, either induces a neutrophilic differentiation block or confers abnormal migration properties in a ligand-dependent manner. *Blood* **104**, 526-534.
- Allalunis-Turner,M.J., Walden,T.L., Jr., and Sawich,C. (1989). Induction of marrow hypoxia by radioprotective agents. *Radiat. Res.* **118**, 581-586.
- Anderlini,P., Przepiorka,D., Seong,C., Smith,T.L., Huh,Y.O., Lauppe,J., Champlin,R., and Korbling,M. (1997). Factors affecting mobilization of CD34+ cells in normal donors treated with filgrastim. *Transfusion* **37**, 507-512.
- Anderlini,P., Przepiorka,D., Korbling,M., and Champlin,R. (1998). Blood stem cell procurement: donor safety issues. *Bone Marrow Transplant.* **21 Suppl 3**, S35-S39.
- Anderlini,P., Rizzo,J.D., Nugent,M.L., Schmitz,N., Champlin,R.E., and Horowitz,M.M. (2001). Peripheral blood stem cell donation: an analysis from the International Bone Marrow Transplant Registry (IBMTR) and European Group for Blood and Marrow Transplant (EBMT) databases. *Bone Marrow Transplant.* **27**, 689-692.
- Andrews,R.G., Singer,J.W., and Bernstein,I.D. (1986). Monoclonal antibody 12-8 recognizes a 115-kd molecule present on both unipotent and multipotent hematopoietic colony-forming cells and their precursors. *Blood* **67**, 842-845.
- Anno,G.H., Baum,S.J., Withers,H.R., and Young,R.W. (1989). Symptomatology of acute radiation effects in humans after exposure to doses of 0.5-30 Gy. *Health Phys.* **56**, 821-838.
- Arai,F., Hirao,A., Ohmura,M., Sato,H., Matsuoka,S., Takubo,K., Ito,K., Koh,G.Y., and Suda,T. (2004). Tie2/angiopoietin-1 signaling regulates hematopoietic stem cell quiescence in the bone marrow niche. *Cell* **118**, 149-161.
- Araki,H., Baluchamy,S., Yoshinaga,K., Petro,B., Petiwala,S., Parajuli,R., Milhem,M., Lavelle,D., DeSimone,J., and Mahmud,N. (2009). Cord blood stem cell expansion is permissive to epigenetic regulation and environmental cues. *Exp. Hematol.* **37**, 1084-1095.

- Asahara,T., Murohara,T., Sullivan,A., Silver,M., van der Zee,R., Li,T., Witzenbichler,B., Schatteman,G., and Isner,J.M. (1997). Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275, 964-967.
- Asahara,T., Takahashi,T., Masuda,H., Kalka,C., Chen,D., Iwaguro,H., Inai,Y., Silver,M., and Isner,J.M. (1999). VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. *EMBO J.* 18, 3964-3972.
- Assmus,B., Schachinger,V., Teupe,C., Britten,M., Lehmann,R., Dobert,N., Grunwald,F., Aicher,A., Urbich,C., Martin,H., Hoelzer,D., Dimmeler,S., and Zeiher,A.M. (2002). Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation* 106, 3009-3017.
- Ates,M., Hamza,M., Seidel,K., Kotalla,C.E., Ledent,C., and Guhring,H. (2003). Intrathecally applied flurbiprofen produces an endocannabinoid-dependent antinociception in the rat formalin test. *Eur. J. Neurosci.* 17, 597-604.
- Aversa,F., Tabilio,A., Velardi,A., Cunningham,I., Terenzi,A., Falzetti,F., Ruggeri,L., Barbabietola,G., Aristei,C., Latini,P., Reisner,Y., and Martelli,M.F. (1998). Treatment of high risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype. *N Eng J Med* 339, 1186-1193.
- Balaguer,H., Galmes,A., Ventayol,G., Bargay,J., and Besalduch,J. (2004). Splenic rupture after granulocyte-colony-stimulating factor mobilization in a peripheral blood progenitor cell donor. *Transfusion* 44, 1260-1261.
- Balazs,A.B., Fabian,A.J., Esmon,C.T., and Mulligan,R.C. (2006). Endothelial protein C receptor (CD201) explicitly identifies hematopoietic stem cells in murine bone marrow. *Blood* 107, 2317-2321.
- Baratelli,F., Krysan,K., Heuze-Vourc'h,N., Zhu,L., Escuadro,B., Sharma,S., Reckamp,K., Dohadwala,M., and Dubinett,S.M. (2005). PGE2 confers survivin-dependent apoptosis resistance in human monocyte-derived dendritic cells. *J. Leukoc. Biol.* 78, 555-564.
- Batinic,D., Tindle,R., Boban,D., Tiefenbach,A., Rajic,L., Labar,B., Nemet,D., and Boranic,M. (1989). Expression of haematopoietic progenitor cell-associated antigen BI-3C5/CD34 in leukemia. *Leuk. Res.* 13, 83-85.
- Baum,C.M., Weissman,I.L., Tsukamoto,A.S., Buckle,A.M., and Peault,B. (1992). Isolation of a candidate human hematopoietic stem-cell population. *Proc. Natl. Acad. Sci. U. S. A* 89, 2804-2808.

Bautz,F., Denzlinger,C., Kanz,L., and Mohle,R. (2001). Chemotaxis and transendothelial migration of CD34(+) hematopoietic progenitor cells induced by the inflammatory mediator leukotriene D4 are mediated by the 7-transmembrane receptor CysLT1. *Blood* 97, 3433-3440.

Becker,A.J., McCulloch,E.A., and Till,J.E. (1963). Cytological demonstration of the clonal nature of spleen colonies derived from transplanted mouse marrow cells. *Nature* 197, 452-454.

Becker,P.S., Wagle,M., Matous,S., Swanson,R.S., Pihan,G., Lowry,P.A., Stewart,F.M., and Heard,S.O. (1997). Spontaneous splenic rupture following administration of granulocyte colony-stimulating factor (G-CSF): occurrence in an allogeneic donor of peripheral blood stem cells. *Biol. Blood Marrow Transplant.* 3, 45-49.

Benito,A.I., Diaz,M.A., Gonzalez-Vicent,M., Sevilla,J., and Madero,L. (2004). Hematopoietic stem cell transplantation using umbilical cord blood progenitors: review of current clinical results. *Bone Marrow Transplant.* 33, 675-690.

Bentzen,P.J. and Lang,F. (2007). Effect of anandamide on erythrocyte survival. *Cell Physiol Biochem.* 20, 1033-1042.

Benveniste,P., Cantin,C., Hyam,D., and Iscove,N.N. (2003). Hematopoietic stem cells engraft in mice with absolute efficiency. *Nat. Immunol.* 4, 708-713.

Benveniste,P., Frelin,C., Janmohamed,S., Barbara,M., Herrington,R., Hyam,D., and Iscove,N.N. (2010). Intermediate-term hematopoietic stem cells with extended but time-limited reconstitution potential. *Cell Stem Cell* 6, 48-58.

Berenson,R.J., Andrews,R.G., Bensinger,W.I., Kalamasz,D., Knitter,G., Buckner,C.D., and Bernstein,I.D. (1988). Antigen CD34+ marrow cells engraft lethally irradiated baboons. *J. Clin. Invest* 81, 951-955.

Bergstrom,S. (1967). Prostaglandins: members of a new hormonal system. These physiologically very potent compounds of ubiquitous occurrence are formed from essential fatty acids. *Science* 157, 382-391.

Bertolini,A., Ottani,A., and Sandrini,M. (2002). Selective COX-2 inhibitors and dual acting anti-inflammatory drugs: critical remarks. *Curr. Med. Chem.* 9, 1033-1043.

Bhatia,M., Wang,J.C., Kapp,U., Bonnet,D., and Dick,J.E. (1997). Purification of primitive human hematopoietic cells capable of repopulating immune-deficient mice. *Proc. Natl. Acad. Sci. U. S. A* 94, 5320-5325.

Bhatia,M., Bonnet,D., Murdoch,B., Gan,O.I., and Dick,J.E. (1998). A newly discovered class of human hematopoietic cells with SCID-repopulating activity. *Nat. Med.* 4, 1038-1045.

Bhattacharya,D., Czechowicz,A., Ooi,A.G., Rossi,D.J., Bryder,D., and Weissman,I.L. (2009). Niche recycling through division-independent egress of hematopoietic stem cells. *J. Exp. Med.* 206, 2837-2850.

Bittencourt,H., Rocha,V., Chevret,S., Socie,G., Esperou,H., Devergie,A., Dal,C.L., Marolleau,J.P., Garnier,F., Ribaud,P., and Gluckman,E. (2002). Association of CD34 cell dose with hematopoietic recovery, infections, and other outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 99, 2726-2733.

Braccioni,F., Dorman,S.C., O'byrne,P.M., Inman,M.D., Denburg,J.A., Parameswaran,K., Baatjes,A.J., Foley,R., and Gauvreau,G.M. (2002). The effect of cysteinyl leukotrienes on growth of eosinophil progenitors from peripheral blood and bone marrow of atopic subjects. *J. Allergy Clin. Immunol.* 110, 96-101.

Bradley,T.R. and Metcalf,D. (1966). The growth of mouse bone marrow cells in vitro. *Aust. J. Exp. Biol. Med. Sci.* 44, 287-299.

Brandt,J., Baird,N., Lu,L., Srour,E., and Hoffman,R. (1988). Characterization of a human hematopoietic progenitor cell capable of forming blast cell containing colonies in vitro. *J. Clin. Invest* 82, 1017-1027.

Brenner,S., Whiting-Theobald,N., Kawai,T., Linton,G.F., Rudikoff,A.G., Choi,U., Ryser,M.F., Murphy,P.M., Sechler,J.M., and Malech,H.L. (2004). CXCR4-transgene expression significantly improves marrow engraftment of cultured hematopoietic stem cells. *Stem Cells* 22, 1128-1133.

Breyer,R.M., Bagdassarian,C.K., Myers,S.A., and Breyer,M.D. (2001). Prostanoid receptors: subtypes and signaling. *Annu. Rev. Pharmacol. Toxicol.* 41, 661-690.

Brown,G., Bunce,C.M., and Guy,G.R. (1985). Sequential determination of lineage potentials during haemopoiesis. *Br. J. Cancer* 52, 681-686.

Brown,G., Hughes,P.J., Michell,R.H., Rolink,A.G., and Ceredig,R. (2007). The sequential determination model of hematopoiesis. *Trends Immunol.* 28, 442-448.

Broxmeyer,H.E., Galbraith,P.R., and Baker,F.L. (1976). Relationship of colony-stimulating activity to apparent kill of human colony-forming cells by irradiation and hydroxyurea. *Blood* 47, 403-411.

Broxmeyer,H.E., Cooper,S., and Gabig,T. (1989). The effects of oxidizing species derived from molecular oxygen on the proliferation in vitro of human granulocyte-macrophage progenitor cells. *Ann. N. Y. Acad. Sci.* 554, 177-184.

Broxmeyer,H.E., Cooper,S., Lu,L., Miller,M.E., Langefeld,C.D., and Ralph,P. (1990). Enhanced stimulation of human bone marrow macrophage colony formation in vitro by recombinant human macrophage colony-stimulating factor in agarose medium and at low oxygen tension. *Blood* 76, 323-329.

Broxmeyer,H.E., Kurtzberg,J., Gluckman,E., Auerbach,A.D., Douglas,G., Cooper,S., Falkenburg,J.H., Bard,J., and Boyse,E.A. (1991). Umbilical cord blood hematopoietic stem and repopulating cells in human clinical transplantation. *Blood Cells* 17, 313-329.

Broxmeyer,H.E. and Cooper,S. (1997). High-efficiency recovery of immature haematopoietic progenitor cells with extensive proliferative capacity from human cord blood cryopreserved for 10 years. *Clin. Exp. Immunol.* 107 Suppl 1, 45-53.

Broxmeyer,H.E., Kohli,L., Kim,C.H., Lee,Y., Mantel,C., Cooper,S., Hangoc,G., Shaheen,M., Li,X., and Clapp,D.W. (2003). Stromal cell-derived factor-1/CXCL12 directly enhances survival/antiapoptosis of myeloid progenitor cells through CXCR4 and G(alpha)i proteins and enhances engraftment of competitive, repopulating stem cells. *J. Leukoc. Biol.* 73, 630-638.

Broxmeyer,H.E. (2005). Biology of cord blood cells and future prospects for enhanced clinical benefit. *Cytotherapy.* 7, 209-218.

Broxmeyer,H.E., Cooper,S., Hangoc,G., and Kim,C.H. (2005a). Stromal cell-derived factor-1/CXCL12 selectively counteracts inhibitory effects of myelosuppressive chemokines on hematopoietic progenitor cell proliferation in vitro. *Stem Cells Dev.* 14, 199-203.

Broxmeyer,H.E., Orschell,C.M., Clapp,D.W., Hangoc,G., Cooper,S., Plett,P.A., Liles,W.C., Li,X., Graham-Evans,B., Campbell,T.B., Calandra,G., Bridger,G., Dale,D.C., and Srour,E.F. (2005b). Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J. Exp. Med.* 201, 1307-1318.

Broxmeyer,H.E. (2006). Cord Blood Hematopoietic Stem and Progenitor Cells. In *Essentials of Stem Cell Biology*, Elsevier, Inc.), pp. 133-137.

Broxmeyer,H.E., Srour,E., Orschell,C., Ingram,D.A., Cooper,S., Plett,P.A., Mead,L.E., and Yoder,M.C. (2006). Cord blood stem and progenitor cells. *Methods Enzymol.* 419, 439-473.

- Broxmeyer,H.E., Mejia,J.A., Hangoc,G., Barese,C., Dinauer,M., and Cooper,S. (2007a). SDF-1/CXCL12 enhances in vitro replating capacity of murine and human multipotential and macrophage progenitor cells. *Stem Cells Dev.* 16, 589-596.
- Broxmeyer,H.E., Hangoc,G., Cooper,S., Campbell,T., Ito,S., and Mantel,C. (2007b). AMD3100 and CD26 modulate mobilization, engraftment, and survival of hematopoietic stem and progenitor cells mediated by the SDF-1/CXCL12-CXCR4 axis. *Ann. N. Y. Acad. Sci.* 1106, 1-19.
- Broxmeyer,H.E. and Smith,S. (2009). Cord blood stem cell transplantation. In Thomas' Hematopoietic Cell Transplantation, F.R.Appelbaum, S.J.Forman, R.S.Negrin, and K.G.Blume, eds. (Oxford: Wiley-Blackwell), pp. 559-576.
- Burr,G.O. and Burr,M.M. (1930). On the Nature and Role of the Fatty Acids Essential in Nutrition. *Journal of Biological Chemistry* 86, 587-621.
- Burstein,S.H., Rossetti,R.G., Yagen,B., and Zurier,R.B. (2000). Oxidative metabolism of anandamide. *Prostaglandins Other Lipid Mediat.* 61, 29-41.
- Cadigan,K.M. and Nusse,R. (1997). Wnt signaling: a common theme in animal development. *Genes Dev.* 11, 3286-3305.
- Calvi,L.M., Adams,G.B., Weibrecht,K.W., Weber,J.M., Olson,D.P., Knight,M.C., Martin,R.P., Schipani,E., Divieti,P., Bringham,F.R., Milner,L.A., Kronenberg,H.M., and Scadden,D.T. (2003). Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature* 425, 841-846.
- Cayeux,S.J., Beverley,P.C., Schulz,R., and Dorken,B. (1993). Elevated plasma prostaglandin E2 levels found in 14 patients undergoing autologous bone marrow or stem cell transplantation. *Bone Marrow Transplant* 12, 603-608.
- Chang,J.K., Wang,G.J., Tsai,S.T., and Ho,M.L. (2005). Nonsteroidal anti-inflammatory drug effects on osteoblastic cell cycle, cytotoxicity, and cell death. *Connect. Tissue Res.* 46, 200-210.
- Chen,C.Z., Li,M., de,G.D., Monti,S., Gottgens,B., Sanchez,M.J., Lander,E.S., Golub,T.R., Green,A.R., and Lodish,H.F. (2002). Identification of endoglin as a functional marker that defines long-term repopulating hematopoietic stem cells. *Proc. Natl. Acad. Sci. U. S. A* 99, 15468-15473.
- Chen,C.Z., Li,L., Li,M., and Lodish,H.F. (2003). The endoglin(positive) sca-1(positive) rhodamine(low) phenotype defines a near-homogeneous population of long-term repopulating hematopoietic stem cells. *Immunity.* 19, 525-533.

Chen,J., Ellison,F.M., Keyvanfar,K., Omokaro,S.O., Desierto,M.J., Eckhaus,M.A., and Young,N.S. (2008). Enrichment of hematopoietic stem cells with SLAM and LSK markers for the detection of hematopoietic stem cell function in normal and Trp53 null mice. *Exp. Hematol.* 36, 1236-1243.

Chen,Q.R., Miyaura,C., Higashi,S., Murakami,M., Kudo,I., Saito,S., Hiraide,T., Shibasaki,Y., and Suda,T. (1997). Activation of cytosolic phospholipase A2 by platelet-derived growth factor is essential for cyclooxygenase-2-dependent prostaglandin E2 synthesis in mouse osteoblasts cultured with interleukin-1. *J Biol Chem* 272, 5952-5958.

Chen,X., Gresham,A., Morrison,A., and Pentland,A.P. (1996). Oxidative stress mediates synthesis of cytosolic phospholipase A2 after UVB injury. *Biochim Biophys Acta* 1299, 23-33.

Cheng,T., Rodrigues,N., Shen,H., Yang,Y., Dombkowski,D., Sykes,M., and Scadden,D.T. (2000). Hematopoietic stem cell quiescence maintained by p21cip1/waf1. *Science* 287, 1804-1808.

Chervenick,P.A. and Boggs,D.R. (1971). In vitro growth of granulocytic and mononuclear cell colonies from blood of normal individuals. *Blood* 37, 131-135.

Chinsoo,C.L. and Glatstein,E. (1998). Radiation Injury. In *Harrison's Principles of Internal Medicine*, A.S.Fauci, E.Braunwald, and K.L.Isselbacher, eds. (New York: McGraw-Hill), p. 2559.

Christensen,J.L. and Weissman,I.L. (2001). Flk-2 is a marker in hematopoietic stem cell differentiation: a simple method to isolate long-term stem cells. *Proc. Natl. Acad. Sci. U. S. A* 98, 14541-14546.

Christopherson,K.W., Hangoc,G., Mantel,C.R., and Broxmeyer,H.E. (2004). Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science* 305, 1000-1003.

Chui,D.H., Liao,S.K., and Walker,K. (1978). Fetal erythropoiesis in steel mutant mice. III. Defect in differentiation from BFU-E to CFU-E during early development. *Blood* 51, 539-547.

Chung,J.W., Kim,G.Y., Mun,Y.C., Ahn,J.Y., Seong,C.M., and Kim,J.H. (2005). Leukotriene B4 pathway regulates the fate of the hematopoietic stem cells. *Exp. Mol. Med* 37, 45-50.

Chung,Y.S., Kim,H.J., Kim,T.M., Hong,S.H., Kwon,K.R., An,S., Park,J.H., Lee,S., and Oh,I.H. (2009). Undifferentiated hematopoietic cells are characterized by a genome-wide undermethylation dip around the transcription start site and a hierarchical epigenetic plasticity. *Blood* 114, 4968-4978.

Civin,C.I., Strauss,L.C., Brovall,C., Fackler,M.J., Schwartz,J.F., and Shaper,J.H. (1984). Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. *J. Immunol.* 133, 157-165.

Civin,C.I., Almeida-Porada,G., Lee,M.J., Olweus,J., Terstappen,L.W., and Zanjani,E.D. (1996). Sustained, retransplantable, multilineage engraftment of highly purified adult human bone marrow stem cells in vivo. *Blood* 88, 4102-4109.

Claesson,H.E., Dahlberg,N., and Gahrton,G. (1985). Stimulation of human myelopoiesis by leukotriene B4. *Biochem. Biophys. Res. Commun.* 131, 579-585.

Clark,J.D., Schievella,A.R., Nalefski,E.A., and Lin,L.L. (1995). Cytosolic phospholipase A2. *J. Lipid Mediat. Cell Signal.* 12, 83-117.

Coffman,R.L. and Weissman,I.L. (1981). A monoclonal antibody that recognizes B cells and B cell precursors in mice. *J. Exp. Med.* 153, 269-279.

Coleman,C.N., Blakely,W.F., Fike,J.R., MacVittie,T.J., Metting,N.F., Mitchell,J.B., Moulder,J.E., Preston,R.J., Seed,T.M., Stone,H.B., Tofilon,P.J., and Wong,R.S. (2003). Molecular and cellular biology of moderate-dose (1-10 Gy) radiation and potential mechanisms of radiation protection: report of a workshop at Bethesda, Maryland, December 17-18, 2001. *Radiat. Res.* 159, 812-834.

Conneally,E., Cashman,J., Petzer,A., and Eaves,C. (1997). Expansion in vitro of transplantable human cord blood stem cells demonstrated using a quantitative assay of their lympho-myeloid repopulating activity in nonobese diabetic-scid/scid mice. *Proc. Natl. Acad. Sci. U. S. A* 94, 9836-9841.

Coopman,K., Smith,L.D., Wright,K.L., and Ward,S.G. (2007). Temporal variation in CB2R levels following T lymphocyte activation: evidence that cannabinoids modulate CXCL12-induced chemotaxis. *Int. Immunopharmacol.* 7, 360-371.

Cormier-Regard,S., Nguyen,S.V., and Claycomb,W.C. (1998). Adrenomedullin gene expression is developmentally regulated and induced by hypoxia in rat ventricular cardiac myocytes. *J. Biol. Chem.* 273, 17787-17792.

Coruzzi,G., Venturi,N., and Spaggiari,S. (2007). Gastrointestinal safety of novel nonsteroidal antiinflammatory drugs: selective COX-2 inhibitors and beyond. *Acta Biomed.* 78, 96-110.

Couban,S., Simpson,D.R., Barnett,M.J., Bredeson,C., Hubesch,L., Howson-Jan,K., Shore,T.B., Walker,I.R., Browett,P., Messner,H.A., Panzarella,T., and Lipton,J.H. (2002). A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplants for myeloid malignancies. *Blood* 100, 1525-1531.

Croxford,J.L. and Yamamura,T. (2005). Cannabinoids and the immune system: potential for the treatment of inflammatory diseases? *J. Neuroimmunol.* 166, 3-18.

Cui,J., Wahl,R.L., Shen,T., Fisher,S.J., Recker,E., Ginsburg,D., and Long,M.W. (1999). Bone marrow cell trafficking following intravenous administration. *Br. J. Haematol.* 107, 895-902.

Cutler,C., Giri,S., Jeyapalan,S., Paniagua,D., Viswanathan,A., and Antin,J.H. (2001). Acute and chronic graft-versus-host disease after allogeneic peripheral-blood stem-cell and bone marrow transplantation: a meta-analysis. *J. Clin. Oncol.* 19, 3685-3691.

Dainiak,N., Waselenko,J.K., Armitage,J.O., MacVittie,T.J., and Farese,A.M. (2003). The hematologist and radiation casualties. *Hematology. Am. Soc. Hematol. Educ. Program.* 473-496.

Danet,G.H., Pan,Y., Luongo,J.L., Bonnet,D.A., and Simon,M.C. (2003). Expansion of human SCID-repopulating cells under hypoxic conditions. *J. Clin. Invest* 112, 126-135.

Dao,M.A., Arevalo,J., and Nolta,J.A. (2003). Reversibility of CD34 expression on human hematopoietic stem cells that retain the capacity for secondary reconstitution. *Blood* 101, 112-118.

Davis,B.J., Lennard,D.E., Lee,C.A., Tiano,H.F., Morham,S.G., Wetsel,W.C., and Langenbach,R. (1999). Anovulation in cyclooxygenase-2-deficient mice is restored by prostaglandin E2 and interleukin-1beta. *Endocrinology* 140, 2685-2695.

Desai,S., April,H., Nwaneshiudu,C., and Ashby,B. (2000). Comparison of agonist-induced internalization of the human EP2 and EP4 prostaglandin receptors: role of the carboxyl terminus in EP4 receptor sequestration. *Mol. Pharmacol.* 58, 1279-1286.

Desouza,I.A., Franco-Penteado,C.F., Camargo,E.A., Lima,C.S., Teixeira,S.A., Muscara,M.N., De,N.G., and Antunes,E. (2005). Inflammatory mechanisms underlying the rat pulmonary neutrophil influx induced by airway exposure to staphylococcal enterotoxin type A. *Br. J. Pharmacol.* 146, 781-791.

Devine,S.M., Brown,R.A., Mathews,V., Trinkaus,K., Khoury,H., Adkins,D., Vij,R., Sempek,D., Graubert,T., Tomasson,M., Goodnough,L.T., and DiPersio,J.F. (2005). Reduced risk of acute GVHD following mobilization of HLA-identical sibling donors with GM-CSF alone. *Bone Marrow Transplant.* 36, 531-538.

Devine,S.M., Vij,R., Rettig,M., Todt,L., McGlauchlen,K., Fisher,N., Devine,H., Link,D.C., Calandra,G., Bridger,G., Westervelt,P., and DiPersio,J.F. (2008). Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. *Blood* 112, 990-998.

Dexter,T.M., Allen,T.D., and Lajtha,L.G. (1977). Conditions controlling the proliferation of haemopoietic stem cells in vitro. *J. Cell Physiol* 91, 335-344.

Dick,J.E., Sirard,C., Pflumio,F., and Lapidot,T. (1992). Murine models of normal and neoplastic human haematopoiesis. *Cancer Surv.* 15, 161-181.

Dieter,P., Scheibe,R., Kamionka,S., and Kolada,A. (2002). LPS-induced synthesis and release of PGE2 in liver macrophages: regulation by CPLA2, COX-1, COX-2, and PGE2 synthase. *Adv. Exp. Med. Biol.* 507, 457-462.

Dimmeler,S., Zeiher,A.M., and Schneider,M.D. (2005). Unchain my heart: the scientific foundations of cardiac repair. *J. Clin. Invest* 115, 572-583.

Domen,J., Cheshier,S.H., and Weissman,I.L. (2000). The role of apoptosis in the regulation of hematopoietic stem cells: Overexpression of Bcl-2 increases both their number and repopulation potential. *J. Exp. Med* 191, 253-264.

Dominici,M., Rasini,V., Bussolari,R., Chen,X., Hofmann,T.J., Spano,C., Bernabei,D., Veronesi,E., Bertoni,F., Paolucci,P., Conte,P., and Horwitz,E.M. (2009). Restoration and reversible expansion of the osteoblastic hematopoietic stem cell niche after marrow radioablation. *Blood* 114, 2333-2343.

Donnelly,D.S. and Krause,D.S. (2001). Hematopoietic stem cells can be CD34+ or CD34-. *Leuk. Lymphoma* 40, 221-234.

Dorrell,C., Gan,O.I., Pereira,D.S., Hawley,R.G., and Dick,J.E. (2000). Expansion of human cord blood CD34(+)CD38(-) cells in ex vivo culture during retroviral transduction without a corresponding increase in SCID repopulating cell (SRC) frequency: dissociation of SRC phenotype and function. *Blood* 95, 102-110.

Duffield-Lillico,A.J., Boyle,J.O., Zhou,X.K., Ghosh,A., Butala,G.S., Subbaramaiah,K., Newman,R.A., Morrow,J.D., Milne,G.L., and Dannenberg,A.J. (2009). Levels of prostaglandin E metabolite and leukotriene E(4) are increased in the urine of smokers: evidence that celecoxib shunts arachidonic acid into the 5-lipoxygenase pathway. *Cancer Prev. Res. (Phila Pa)* 2, 322-329.

- Duhrsen,U., Villeval,J.L., Boyd,J., Kannourakis,G., Morstyn,G., and Metcalf,D. (1988). Effects of recombinant human granulocyte colony-stimulating factor on hematopoietic progenitor cells in cancer patients. *Blood* 72, 2074-2081.
- Dupuis,F., Desplat,V., Praloran,V., and Denizot,Y. (1997). Effects of lipidic mediators on the growth of human myeloid and erythroid marrow progenitors. *J. Lipid Mediat. Cell Signal.* 16, 117-125.
- El Badri,N.S., Wang,B.Y., Cherry, and Good,R.A. (1998). Osteoblasts promote engraftment of allogeneic hematopoietic stem cells. *Exp Hematol* 26, 110-116.
- Elsas,P.X., Queto,T., Mendonca-Sales,S.C., Elsas,M.I., Kanaoka,Y., and Lam,B.K. (2008). Cysteinyl leukotrienes mediate the enhancing effects of indomethacin and aspirin on eosinophil production in murine bone marrow cultures. *Br. J. Pharmacol.* 153, 528-535.
- Estrov,Z., Halperin,D.S., Coceani,F., and Freedman,M.H. (1988). Modulation of human marrow haematopoiesis by leucotrienes in vitro. *Br. J. Haematol.* 69, 321-327.
- Evans,J.H., Spencer,D.M., Zweifach,A., and Leslie,C.C. (2001). Intracellular calcium signals regulating cytosolic phospholipase A2 translocation to internal membranes. *J. Biol. Chem.* 276, 30150-30160.
- Falzetti,F., Aversa,F., Minelli,O., and Tabilio,A. (1999). Spontaneous rupture of spleen during peripheral blood stem-cell mobilisation in a healthy donor. *Lancet* 353, 555.
- Fauser,A.A. and Messner,H.A. (1978). Granuloerythropoietic colonies in human bone marrow, peripheral blood, and cord blood. *Blood* 52, 1243-1248.
- Fauser,A.A. and Messner,H.A. (1979). Identification of megakaryocytes, macrophages, and eosinophils in colonies of human bone marrow containing neutrophilic granulocytes and erythroblasts. *Blood* 53, 1023-1027.
- Feher,I. and Gidali,J. (1974). Prostaglandin E2 as stimulator of haemopoietic stem cell proliferation. *Nature* 247, 550-551.
- Feldser,D., Agani,F., Iyer,N.V., Pak,B., Ferreira,G., and Semenza,G.L. (1999). Reciprocal positive regulation of hypoxia-inducible factor 1alpha and insulin-like growth factor 2. *Cancer Res.* 59, 3915-3918.
- Fernandez-Martinez,A., Molla,B., Mayoral,R., Bosca,L., Casado,M., and Martin-Sanz,P. (2006). Cyclo-oxygenase 2 expression impairs serum-withdrawal-induced apoptosis in liver cells. *Biochem. J.* 398, 371-380.

Flake,A.W., Harrison,M.R., Adzick,N.S., and Zanjani,E.D. (1986). Transplantation of fetal hematopoietic stem cells in utero: the creation of hematopoietic chimeras. *Science* 233, 776-778.

Fleming,H.E., Janzen,V., Lo,C.C., Guo,J., Leahy,K.M., Kronenberg,H.M., and Scadden,D.T. (2008). Wnt signaling in the niche enforces hematopoietic stem cell quiescence and is necessary to preserve self-renewal in vivo. *Cell Stem Cell* 2, 274-283.

Flower,R.J. (1990). Lipocortin. *Prog. Clin. Biol. Res.* 349, 11-25.

Flower,R.J. (2006). Prostaglandins, bioassay and inflammation. *Br. J. Pharmacol.* 147 Suppl 1, S182-S192.

Folco,G. and Murphy,R.C. (2006). Eicosanoid transcellular biosynthesis: from cell-cell interactions to in vivo tissue responses. *Pharmacol. Rev.* 58, 375-388.

Ford,C.E., Hamerton,J.L., Barnes,D.W., and Loutit,J.F. (1956). Cytological identification of radiation-chimaeras. *Nature* 177, 452-454.

Forde,S., Tye,B.J., Newey,S.E., Roubelakis,M., Smythe,J., McGuckin,C.P., Pettengell,R., and Watt,S.M. (2007). Endolyn (CD164) modulates the CXCL12-mediated migration of umbilical cord blood CD133+ cells. *Blood* 109, 1825-1833.

Fortanier,C., Kuentz,M., Sutton,L., Milpied,N., Michalet,M., Macquart-Moulin,G., Faucher,C., Le Corroller,A.G., Moatti,J.P., and Blaise,D. (2002). Healthy sibling donor anxiety and pain during bone marrow or peripheral blood stem cell harvesting for allogeneic transplantation: results of a randomised study. *Bone Marrow Transplant.* 29, 145-149.

Fowler,C.J. (2004). Possible involvement of the endocannabinoid system in the actions of three clinically used drugs. *Trends Pharmacol. Sci.* 25, 59-61.

Freimark,D., Matetzky,S., Leor,J., Boyko,V., Barbash,I.M., Behar,S., and Hod,H. (2002). Timing of aspirin administration as a determinant of survival of patients with acute myocardial infarction treated with thrombolysis. *Am. J. Cardiol.* 89, 381-385.

Frenette,P.S., Subbarao,S., Mazo,I.B., von Andrian,U.H., and Wagner,D.D. (1998). Endothelial selectins and vascular cell adhesion molecule-1 promote hematopoietic progenitor homing to bone marrow. *Proc. Natl. Acad. Sci. U. S. A* 95, 14423-14428.

Frenette,P.S. and Weiss,L. (2000). Sulfated glycans induce rapid hematopoietic progenitor cell mobilization: evidence for selectin-dependent and independent mechanisms. *Blood* 96, 2460-2468.

- Fruehauf, S. and Seggewiss, R. (2003). It's moving day: factors affecting peripheral blood stem cell mobilization and strategies for improvement [corrected]. *Br. J. Haematol.* 122, 360-375.
- Fujino, H., Xu, W., and Regan, J.W. (2003). Prostaglandin E2 induced functional expression of early growth response factor-1 by EP4, but not EP2, prostanoid receptors via the phosphatidylinositol 3-kinase and extracellular signal-regulated kinases. *J. Biol. Chem.* 278, 12151-12156.
- Fukuda, R., Kelly, B., and Semenza, G.L. (2003). Vascular endothelial growth factor gene expression in colon cancer cells exposed to prostaglandin E2 is mediated by hypoxia-inducible factor 1. *Cancer Res.* 63, 2330-2334.
- Fukuda, S. and Pelus, L.M. (2001). Regulation of the inhibitor-of-apoptosis family member survivin in normal cord blood and bone marrow CD34(+) cells by hematopoietic growth factors: implication of survivin expression in normal hematopoiesis. *Blood* 98, 2091-2100.
- Fukuda, S. and Pelus, L.M. (2002). Elevation of Survivin levels by hematopoietic growth factors occurs in quiescent CD34+ hematopoietic stem and progenitor cells before cell cycle entry. *Cell Cycle* 1, 322-326.
- Fukuda, S., Foster, R.G., Porter, S.B., and Pelus, L.M. (2002). The antiapoptosis protein survivin is associated with cell cycle entry of normal cord blood CD34(+) cells and modulates cell cycle and proliferation of mouse hematopoietic progenitor cells. *Blood* 100, 2463-2471.
- Fukuda, S., Mantel, C.R., and Pelus, L.M. (2004). Survivin regulates hematopoietic progenitor cell proliferation through p21WAF1/Cip1-dependent and -independent pathways. *Blood* 103, 120-127.
- Fukuda, S. and Pelus, L.M. (2006). Internal tandem duplication of Flt3 modulates chemotaxis and survival of hematopoietic cells by SDF1alpha but negatively regulates marrow homing in vivo. *Exp. Hematol.* 34, 1041-1051.
- Fukuda, S., Bian, H., King, A.G., and Pelus, L.M. (2007). The chemokine GRObeta mobilizes early hematopoietic stem cells characterized by enhanced homing and engraftment. *Blood* 110, 860-869.
- Funk, C.D. (2001). Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science* 294, 1871-1875.
- Funk, C.D. (2006). Lipoxygenase pathways as mediators of early inflammatory events in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* 26, 1204-1206.

Gandy,K.L., Domen,J., Aguila,H., and Weissman,I.L. (1999). CD8+TCR+ and CD8+TCR- cells in whole bone marrow facilitate the engraftment of hematopoietic stem cells across allogeneic barriers. *Immunity*. 11, 579-590.

Gentile,P.S., Byer,D., and Pelus,L.M. (1983). In vivo modulation of murine myelopoiesis following intravenous administration of prostaglandin E2. *Blood* 62, 1100-1107.

Gentile,P.S. and Pelus,L.M. (1987). In vivo modulation of myelopoiesis by prostaglandin E2. II. Inhibition of granulocyte-monocyte progenitor cell (CFU-GM) cell-cycle rate. *Exp. Hematol.* 15, 119-126.

Gentile,P.S. and Pelus,L.M. (1988). In vivo modulation of myelopoiesis by prostaglandin E2. IV. Prostaglandin E2 induction of myelopoietic inhibitory activity. *J. Immunol.* 141, 2714-2720.

George,R.J., Sturmoski,M.A., Anant,S., and Houchen,C.W. (2007). EP4 mediates PGE2 dependent cell survival through the PI3 kinase/AKT pathway. *Prostaglandins Other Lipid Mediat.* 83, 112-120.

Gerson,S.L., Talbot,G.H., Hurwitz,S., Strom,B.L., Lusk,E.J., and Cassileth,P.A. (1984). Prolonged granulocytopenia: the major risk factor for invasive pulmonary aspergillosis in patients with acute leukemia. *Ann. Intern. Med.* 100, 345-351.

Ghosh,S., Preet,A., Groopman,J.E., and Ganju,R.K. (2006). Cannabinoid receptor CB2 modulates the CXCL12/CXCR4-mediated chemotaxis of T lymphocytes. *Mol. Immunol.* 43, 2169-2179.

Gidali,J. and Feher,I. (1977). The effect of E type prostaglandins on the proliferation of haemopoietic stem cells in vivo. *Cell Tissue Kinet.* 10, 365-373.

Giet,O., Huygen,S., Beguin,Y., and Gothot,A. (2001). Cell cycle activation of hematopoietic progenitor cells increases very late antigen-5-mediated adhesion to fibronectin. *Exp. Hematol.* 29, 515-524.

Glimm,H., Oh,I.H., and Eaves,C.J. (2000). Human hematopoietic stem cells stimulated to proliferate in vitro lose engraftment potential during their S/G(2)/M transit and do not reenter G(0). *Blood* 96, 4185-4193.

Glover,D., Negendank,W., Delivoria-Papadopoulos,M., and Glick,J.H. (1984). Alterations in oxygen transport following WR-2721. *Int. J. Radiat. Oncol. Biol. Phys.* 10, 1565-1568.

- Gluckman,E., Broxmeyer,H.A., Auerbach,A.D., Friedman,H.S., Douglas,G.W., Devergie,A., Esperou,H., Thierry,D., Socie,G., Lehn,P., and . (1989). Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. *N. Engl. J. Med.* 321, 1174-1178.
- Goetzl,E.J., An,S., and Smith,W.L. (1995). Specificity of expression and effects of eicosanoid mediators in normal physiology and human diseases. *FASEB J.* 9, 1051-1058.
- Goichberg,P., Kalinkovich,A., Borodovsky,N., Tesio,M., Petit,I., Nagler,A., Hardan,I., and Lapidot,T. (2006). cAMP-induced PKCzeta activation increases functional CXCR4 expression on human CD34+ hematopoietic progenitors. *Blood* 107, 870-879.
- Gold,E.J., Conjalka,M., Pelus,L.M., Jhanwar,S.C., Broxmeyer,H., Middleton,A.B., Clarkson,B.D., and Moore,M.A. (1983). Marrow cytogenetic and cell-culture analyses of the myelodysplastic syndromes: insights to pathophysiology and prognosis. *J. Clin. Oncol.* 1, 627-634.
- Goldblatt,M.W. (1935). Properties of human seminal plasma. *J. Physiol* 84, 208-218.
- Goldman,J.M. and Horowitz,M.M. (2002). The international bone marrow transplant registry. *Int. J. Hematol.* 76 *Suppl* 1, 393-397.
- Gong,J.K. (1978). Endosteal marrow: a rich source of hematopoietic stem cells. *Science* 199, 1443-1445.
- Goodman,J.W. and Hodgson,G.S. (1962). Evidence for stem cells in the peripheral blood of mice. *Blood* 19, 702-714.
- Gothot,A., van der Loo,J.C., Clapp,D.W., and Srour,E.F. (1998). Cell cycle-related changes in repopulating capacity of human mobilized peripheral blood CD34(+) cells in non-obese diabetic/severe combined immune-deficient mice. *Blood* 92, 2641-2649.
- Graf,L., Heimfeld,S., and Torok-Storb,B. (2001). Comparison of gene expression in CD34+ cells from bone marrow and G-CSF-mobilized peripheral blood by high-density oligonucleotide array analysis. *Biol. Blood Marrow Transplant.* 7, 486-494.

Grassinger,J., Haylock,D.N., Storan,M.J., Haines,G.O., Williams,B., Whitty,G.A., Vinson,A.R., Be,C.L., Li,S., Sorensen,E.S., Tam,P.P., Denhardt,D.T., Sheppard,D., Choong,P.F., and Nilsson,S.K. (2009). Thrombin-cleaved osteopontin regulates hemopoietic stem and progenitor cell functions through interactions with alpha9beta1 and alpha4beta1 integrins. *Blood* 114, 49-59.

Gratama,J.W., Orfao,A., Barnett,D., Brando,B., Huber,A., Janossy,G., Johnsen,H.E., Keeney,M., Marti,G.E., Preijers,F., Rothe,G., Serke,S., Sutherland,D.R., van der Schoot,C.E., Schmitz,G., and Papa,S. (1998). Flow cytometric enumeration of CD34+ hematopoietic stem and progenitor cells. European Working Group on Clinical Cell Analysis. *Cytometry* 34, 128-142.

Greenberg,P.L., Nichols,W.C., and Schrier,S.L. (1971). Granulopoiesis in acute myeloid leukemia and preleukemia. *N. Engl. J. Med.* 284, 1225-1232.

Gregory,C.J. (1976). Erythropoietin sensitivity as a differentiation marker in the hemopoietic system: studies of three erythropoietic colony responses in culture. *J. Cell Physiol* 89, 289-301.

Guerrero,A.T., Verri,W.A., Jr., Cunha,T.M., Silva,T.A., Schivo,I.R., Dal-Secco,D., Canetti,C., Rocha,F.A., Parada,C.A., Cunha,F.Q., and Ferreira,S.H. (2008). Involvement of LTB4 in zymosan-induced joint nociception in mice: participation of neutrophils and PGE2. *J. Leukoc. Biol.* 83, 122-130.

Hall,E.J. (2000a). *Radiobiology for the Radiologist*. (Philadelphia: Lippincott).

Hall,E.J. (2000b). Acute effects of total body irradiation. In *Radiobiology for the Radiologist*, E.J.Hall, ed. (Philadelphia: Lippincott, Williams & Wilkins), pp. 124-135.

Hall,K.M., Horvath,T.L., Abonour,R., Cornetta,K., and Srour,E.F. (2006). Decreased homing of retrovirally transduced human bone marrow CD34+ cells in the NOD/SCID mouse model. *Exp. Hematol.* 34, 433-442.

Hanson,W.R. and Ainsworth,E.J. (1985). 16,16-Dimethyl prostaglandin E2 induces radioprotection in murine intestinal and hematopoietic stem cells. *Radiat. Res.* 103, 196-203.

Hanson,W.R. (1987). Radiation protection of murine intestine by WR-2721, 16,16-dimethyl prostaglandin E2, and the combination of both agents. *Radiat. Res.* 111, 361-373.

Hara,H. and Ogawa,M. (1978). Murine hemopoietic colonies in culture containing normoblasts, macrophages, and megakaryocytes. *Am. J. Hematol.* 4, 23-34.

- Harizi,H., Grosset,C., and Gualde,N. (2003). Prostaglandin E2 modulates dendritic cell function via EP2 and EP4 receptor subtypes. *J. Leukoc. Biol.* 73, 756-763.
- Harris,J. and Freireich,E.J. (1970). In vitro growth of myeloid colonies from bone marrow of patients with acute leukemia in remission. *Blood* 35, 61-63.
- Harrison,D.E. (1972). Lifesparing ability (in lethally irradiated mice) of W-W mouse marrow with no macroscopic colonies. *Radiat. Res.* 52, 553-563.
- Harrison,D.E. (1980). Competitive repopulation: a new assay for long-term stem cell functional capacity. *Blood* 55, 77-81.
- Harrison,D.E., Jordan,C.T., Zhong,R.K., and Astle,C.M. (1993). Primitive hemopoietic stem cells: direct assay of most productive populations by competitive repopulation with simple binomial, correlation and covariance calculations. *Exp. Hematol.* 21, 206-219.
- Haskill,J.S., McNeill,T.A., and Moore,M.A. (1970). Density distribution analysis of in vivo and in vitro colony forming cells in bone marrow. *J. Cell Physiol* 75, 167-179.
- Hatse,S., Princen,K., Bridger,G., De,C.E., and Schols,D. (2002). Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4. *FEBS Lett.* 527, 255-262.
- Hattori,K., Dias,S., Heissig,B., Hackett,N.R., Lyden,D., Tatenos,M., Hicklin,D.J., Zhu,Z., Witte,L., Crystal,R.G., Moore,M.A., and Rafii,S. (2001). Vascular endothelial growth factor and angiopoietin-1 stimulate postnatal hematopoiesis by recruitment of vasculogenic and hematopoietic stem cells. *J. Exp. Med* 193, 1005-1014.
- Heath,D.S., Axelrad,A.A., McLeod,D.L., and Shreeve,M.M. (1976). Separation of the erythropoietin-responsive progenitors BFU-E and CFU-E in mouse bone marrow by unit gravity sedimentation. *Blood* 47, 777-792.
- Hegen,M., Sun,L., Uozumi,N., Kume,K., Goad,M.E., Nickerson-Nutter,C.L., Shimizu,T., and Clark,J.D. (2003). Cytosolic phospholipase A2alpha-deficient mice are resistant to collagen-induced arthritis. *J. Exp. Med.* 197, 1297-1302.
- Helliwell,R.J., Adams,L.F., and Mitchell,M.D. (2004). Prostaglandin synthases: recent developments and a novel hypothesis. *Prostaglandins Leukot. Essent. Fatty Acids* 70, 101-113.

Herodin,F., Bourin,P., Mayol,J.F., Lataillade,J.J., and Drouet,M. (2003). Short-term injection of antiapoptotic cytokine combinations soon after lethal gamma - irradiation promotes survival. *Blood* 101, 2609-2616.

Herodin,F. and Drouet,M. (2005). Cytokine-based treatment of accidentally irradiated victims and new approaches. *Exp. Hematol.* 33, 1071-1080.

Hill,J.M., Syed,M.A., Arai,A.E., Powell,T.M., Paul,J.D., Zalos,G., Read,E.J., Khuu,H.M., Leitman,S.F., Horne,M., Csako,G., Dunbar,C.E., Waclawiw,M.A., and Cannon,R.O., III (2005). Outcomes and risks of granulocyte colony-stimulating factor in patients with coronary artery disease. *J. Am. Coll. Cardiol.* 46, 1643-1648.

Hillard,C.J. (2000). Biochemistry and pharmacology of the endocannabinoids arachidonylethanolamide and 2-arachidonylglycerol. *Prostaglandins Other Lipid Mediat.* 61, 3-18.

Hofer,M., Pospisil,M., Znojil,V., Hola,J., Vacek,A., Weiterova,L., Streitova,D., and Kozubik,A. (2006). Meloxicam, a cyclooxygenase 2 inhibitor, supports hematopoietic recovery in gamma-irradiated mice. *Radiat. Res.* 166, 556-560.

Hoggatt,J., Singh,P., Sampath,J., and Pelus,L.M. (2009). Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood* 113, 5444-5455.

Holly,R.G. (1955). Studies on iron and cobalt metabolism. *J. Am. Med Assoc.* 158, 1349-1352.

Houchen,C.W., Sturmoski,M.A., Anant,S., Breyer,R.M., and Stenson,W.F. (2003). Prosurvival and antiapoptotic effects of PGE2 in radiation injury are mediated by EP2 receptor in intestine. *Am. J. Physiol Gastrointest. Liver Physiol* 284, G490-G498.

Howlett,A.C. (2002). The cannabinoid receptors. *Prostaglandins Other Lipid Mediat.* 68-69, 619-631.

Hristov,M. and Weber,C. (2006). The therapeutic potential of progenitor cells in ischemic heart disease--Past, present and future. *Basic Res. Cardiol.* 101, 1-7.

Hu,L., Cheng,L., Wang,J., Zhao,H., Duan,H., and Lu,G. (2006). Effects of human yolk sac endothelial cells on supporting expansion of hematopoietic stem/progenitor cells from cord blood. *Cell Biol. Int.* 30, 879-884.

Hu,Z., Zhang,F., Yang,Z., Zhang,J., Zhang,D., Yang,N., Zhang,Y., and Cao,K. (2008). Low-dose aspirin promotes endothelial progenitor cell migration and adhesion and prevents senescence. *Cell Biol. Int.* 32, 761-768.

Huang,S. and Terstappen,L.W. (1994). Lymphoid and myeloid differentiation of single human CD34+, HLA-DR+, CD38- hematopoietic stem cells. *Blood* 83, 1515-1526.

Huang,S.K. and Peters-Golden,M. (2008). Eicosanoid lipid mediators in fibrotic lung diseases: ready for prime time? *Chest* 133, 1442-1450.

Hull,M.A., Ko,S.C., and Hawcroft,G. (2004). Prostaglandin EP receptors: targets for treatment and prevention of colorectal cancer? *Mol. Cancer Ther.* 3, 1031-1039.

Ichikawa,Y., Pluznik,D.H., and Sachs,L. (1966). In vitro control of the development of macrophage and granulocyte colonies. *Proc. Natl. Acad. Sci. U. S. A* 56, 488-495.

Ichiyama,T., Kajimoto,M., Hasegawa,M., Hashimoto,K., Matsubara,T., and Furukawa,S. (2007). Cysteinyl leukotrienes enhance tumour necrosis factor-alpha-induced matrix metalloproteinase-9 in human monocytes/macrophages. *Clin. Exp. Allergy* 37, 608-614.

Igarashi,H., Gregory,S.C., Yokota,T., Sakaguchi,N., and Kincade,P.W. (2002). Transcription from the RAG1 locus marks the earliest lymphocyte progenitors in bone marrow. *Immunity.* 17, 117-130.

Ihara,A., Wada,K., Yoneda,M., Fujisawa,N., Takahashi,H., and Nakajima,A. (2007). Blockade of leukotriene B4 signaling pathway induces apoptosis and suppresses cell proliferation in colon cancer. *J. Pharmacol. Sci.* 103, 24-32.

Ikebuchi,K., Clark,S.C., Ihle,J.N., Souza,L.M., and Ogawa,M. (1988a). Granulocyte colony-stimulating factor enhances interleukin 3-dependent proliferation of multipotential hemopoietic progenitors. *Proc. Natl. Acad. Sci. U. S. A* 85, 3445-3449.

Ikebuchi,K., Ihle,J.N., Hirai,Y., Wong,G.G., Clark,S.C., and Ogawa,M. (1988b). Synergistic factors for stem cell proliferation: further studies of the target stem cells and the mechanism of stimulation by interleukin-1, interleukin-6, and granulocyte colony-stimulating factor. *Blood* 72, 2007-2014.

Ikuta,K. and Weissman,I.L. (1992). Evidence that hematopoietic stem cells express mouse c-kit but do not depend on steel factor for their generation. *Proc. Natl. Acad. Sci. U. S. A* 89, 1502-1506.

Ingram,D.A., Mead,L.E., Tanaka,H., Meade,V., Fenoglio,A., Mortell,K., Pollok,K., Ferkowicz,M.J., Gilley,D., and Yoder,M.C. (2004). Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 104, 2752-2760.

Iscoe,N.N., Senn,J.S., Till,J.E., and McCulloch,E.A. (1971). Colony formation by normal and leukemic human marrow cells in culture: effect of conditioned medium from human leukocytes. *Blood* 37, 1-5.

Iscoe,N.N. and Sieber,F. (1975). Erythroid progenitors in mouse bone marrow detected by macroscopic colony formation in culture. *Exp. Hematol.* 3, 32-43.

Ishii,J., Natsume,A., Wakabayashi,T., Takeuchi,H., Hasegawa,H., Kim,S.U., and Yoshida,J. (2007). The free-radical scavenger edaravone restores the differentiation of human neural precursor cells after radiation-induced oxidative stress. *Neurosci. Lett.* 423, 225-230.

Isoherranen,K., Punnonen,K., Jansen,C., and Uotila,P. (1999). Ultraviolet irradiation induces cyclooxygenase-2 expression in keratinocytes. *Br J Dermatol* 140, 1017-1022.

Ito,C.Y., Kirouac,D.C., Madlambayan,G.J., Yu,M., Rogers,I., and Zandstra,P.W. (2010). The AC133+CD38-, but not the rhodamine-low, phenotype tracks LTC-IC and SRC function in human cord blood ex vivo expansion cultures. *Blood* 115, 257-260.

Ito,M., Hiramatsu,H., Kobayashi,K., Suzue,K., Kawahata,M., Hioki,K., Ueyama,Y., Koyanagi,Y., Sugamura,K., Tsuji,K., Heike,T., and Nakahata,T. (2002). NOD/SCID/gamma(c)(null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood* 100, 3175-3182.

Ivanov,A.I. and Romanovsky,A.A. (2004). Prostaglandin E2 as a mediator of fever: synthesis and catabolism. *Front Biosci.* 9, 1977-1993.

Izumi,T., Yokomizo,T., Obinata,H., Ogasawara,H., and Shimizu,T. (2002). Leukotriene receptors: classification, gene expression, and signal transduction. *J. Biochem.* 132, 1-6.

Jacobson,L.O., Simmons,E.L., Marks,E.K., Robson,M.J., Bethard,W.F., and Gaston,E.O. (1950). The role of the spleen in radiation injury and recovery. *J. Lab Clin. Med.* 35, 746-770.

Jacobson,L.O., Simmons,E.L., Marks,E.K., and Eldredge,J.H. (1951). Recovery from radiation injury. *Science* 113, 510-511.

Jansen,J., Thompson,E.M., Hanks,S., Greenspan,A.R., Thompson,J.M., Dugan,M.J., and Akard,L.P. (1999). Hematopoietic growth factor after autologous peripheral blood transplantation: comparison of G-CSF and GM-CSF. *Bone Marrow Transplant.* 23, 1251-1256.

Janzen,V., Fleming,H.E., Riedt,T., Karlsson,G., Riese,M.J., Lo,C.C., Reynolds,G., Milne,C.D., Paige,C.J., Karlsson,S., Woo,M., and Scadden,D.T. (2008). Hematopoietic stem cell responsiveness to exogenous signals is limited by caspase-3. *Cell Stem Cell* 2, 584-594.

Jetmore,A., Plett,P.A., Tong,X., Wolber,F.M., Breese,R., Abonour,R., Orschell-Traycoff,C.M., and Srour,E.F. (2002). Homing efficiency, cell cycle kinetics, and survival of quiescent and cycling human CD34(+) cells transplanted into conditioned NOD/SCID recipients. *Blood* 99, 1585-1593.

Jiang,S., Fu,Y., Williams,J., Wood,J., Pandarinathan,L., Avraham,S., Makriyannis,A., Avraham,S., and Avraham,H.K. (2007). Expression and function of cannabinoid receptors CB1 and CB2 and their cognate cannabinoid ligands in murine embryonic stem cells. *PLoS. ONE*. 2, e641.

Jiao,W., Kiang,J.G., Cary,L., Elliott,T.B., Pellmar,T.C., and Ledney,G.D. (2009). COX-2 inhibitors are contraindicated for treatment of combined injury. *Radiat. Res.* 172, 686-697.

Jo,D.Y., Rafii,S., Hamada,T., and Moore,M.A. (2000). Chemotaxis of primitive hematopoietic cells in response to stromal cell-derived factor-1. *J. Clin. Invest* 105, 101-111.

Johnson,G.R. and Metcalf,D. (1977). Pure and mixed erythroid colony formation in vitro stimulated by spleen conditioned medium with no detectable erythropoietin. *Proc. Natl. Acad. Sci. U. S. A* 74, 3879-3882.

Jorda,M.A., Verbakel,S.E., Valk,P.J., Vankan-Berkhoudt,Y.V., Maccarrone,M., Finazzi-Agro,A., Lowenberg,B., and Delwel,R. (2002). Hematopoietic cells expressing the peripheral cannabinoid receptor migrate in response to the endocannabinoid 2-arachidonoylglycerol. *Blood* 99, 2786-2793.

Jung,Y.J., Isaacs,J.S., Lee,S., Trepel,J., and Neckers,L. (2003). IL-1beta-mediated up-regulation of HIF-1alpha via an NFkappaB/COX-2 pathway identifies HIF-1 as a critical link between inflammation and oncogenesis. *FASEB J.* 17, 2115-2117.

Kahn,J., Byk,T., Jansson-Sjostrand,L., Petit,I., Shivtiel,S., Nagler,A., Hardan,I., Deutsch,V., Gazit,Z., Gazit,D., Karlsson,S., and Lapidot,T. (2004). Overexpression of CXCR4 on human CD34+ progenitors increases their proliferation, migration, and NOD/SCID repopulation. *Blood* 103, 2942-2949.

Kaidi,A., Qualtrough,D., Williams,A.C., and Paraskeva,C. (2006). Direct transcriptional up-regulation of cyclooxygenase-2 by hypoxia-inducible factor (HIF)-1 promotes colorectal tumor cell survival and enhances HIF-1 transcriptional activity during hypoxia. *Cancer Res.* 66, 6683-6691.

Kang,E.M., Areman,E.M., David-Ocampo,V., Fitzhugh,C., Link,M.E., Read,E.J., Leitman,S.F., Rodgers,G.P., and Tisdale,J.F. (2002). Mobilization, collection, and processing of peripheral blood stem cells in individuals with sickle cell trait. *Blood* 99, 850-855.

Karanu,F.N., Murdoch,B., Gallacher,L., Wu,D.M., Koremoto,M., Sakano,S., and Bhatia,M. (2000). The notch ligand jagged-1 represents a novel growth factor of human hematopoietic stem cells. *J Exp Med* 192, 1365-1372.

Kargacin,B. and Kostial,K. (1985). Reduction of ⁸⁵Sr, ¹³⁷Cs, ¹³¹I and ¹⁴¹Ce retention in rats by simultaneous oral administration of calcium alginate, ferrihexacyanoferrate(II), KI and Zn-DTPA. *Health Phys.* 49, 859-864.

Katayama,Y., Hidalgo,A., Furie,B.C., Vestweber,D., Furie,B., and Frenette,P.S. (2003). PSGL-1 participates in E-selectin-mediated progenitor homing to bone marrow: evidence for cooperation between E-selectin ligands and alpha4 integrin. *Blood* 102, 2060-2067.

Katayama,Y., Hidalgo,A., Peired,A., and Frenette,P.S. (2004). Integrin alpha4beta7 and its counterreceptor MAdCAM-1 contribute to hematopoietic progenitor recruitment into bone marrow following transplantation. *Blood* 104, 2020-2026.

Katayama,Y., Battista,M., Kao,W.M., Hidalgo,A., Peired,A.J., Thomas,S.A., and Frenette,P.S. (2006). Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell* 124, 407-421.

Kato,M., Nishida,S., Kitasato,H., Sakata,N., and Kawai,S. (2001). Cyclooxygenase-1 and cyclooxygenase-2 selectivity of non-steroidal anti-inflammatory drugs: investigation using human peripheral monocytes. *J. Pharm. Pharmacol.* 53, 1679-1685.

Katsuyama,M., Nishigaki,N., Sugimoto,Y., Morimoto,K., Negishi,M., Narumiya,S., and Ichikawa,A. (1995). The mouse prostaglandin E receptor EP2 subtype: cloning, expression, and northern blot analysis. *FEBS Lett.* 372, 151-156.

Kawamoto,H. and Katsura,Y. (2009). A new paradigm for hematopoietic cell lineages: revision of the classical concept of the myeloid-lymphoid dichotomy. *Trends Immunol.* 30, 193-200.

Ke,Q., Kluz,T., and Costa,M. (2005). Down-regulation of the expression of the FIH-1 and ARD-1 genes at the transcriptional level by nickel and cobalt in the human lung adenocarcinoma A549 cell line. *Int. J. Environ. Res. Public Health* 2, 10-13.

Ke,Q. and Costa,M. (2006). Hypoxia-inducible factor-1 (HIF-1). *Mol. Pharmacol.* 70, 1469-1480.

Kellinsalmi,M., Parikka,V., Risteli,J., Hentunen,T., Leskela,H.V., Lehtonen,S., Selander,K., Vaananen,K., and Lehenkari,P. (2007). Inhibition of cyclooxygenase-2 down-regulates osteoclast and osteoblast differentiation and favours adipocyte formation in vitro. *Eur. J. Pharmacol.* 572, 102-110.

Kennedy,M.J., Davis,J., Passos-Coelho,J., Noga,S.J., Huelskamp,A.M., Ohly,K., and Davidson,N.E. (1993). Administration of human recombinant granulocyte colony-stimulating factor (filgrastim) accelerates granulocyte recovery following high-dose chemotherapy and autologous marrow transplantation with 4-hydroperoxycyclophosphamide-purged marrow in women with metastatic breast cancer. *Cancer Res.* 53, 5424-5428.

Kessinger,A. and Armitage,J.O. (1991). The evolving role of autologous peripheral stem cell transplantation following high-dose therapy for malignancies. *Blood* 77, 211-213.

Khan,N.I. and Bendall,L.J. (2006). Role of WNT signaling in normal and malignant hematopoiesis. *Histol. Histopathol.* 21, 761-774.

Kiel,M.J., Yilmaz,O.H., Iwashita,T., Yilmaz,O.H., Terhorst,C., and Morrison,S.J. (2005). SLAM family receptors distinguish hematopoietic stem and progenitor cells and reveal endothelial niches for stem cells. *Cell* 121, 1109-1121.

Kiel,M.J., Radice,G.L., and Morrison,S.J. (2007). Lack of evidence that hematopoietic stem cells depend on N-cadherin-mediated adhesion to osteoblasts for their maintenance. *Cell Stem Cell* 1, 204-217.

Kiel,M.J. and Morrison,S.J. (2008). Uncertainty in the niches that maintain haematopoietic stem cells. *Nat. Rev. Immunol.* 8, 290-301.

Kiel,M.J., Acar,M., Radice,G.L., and Morrison,S.J. (2009). Hematopoietic stem cells do not depend on N-cadherin to regulate their maintenance. *Cell Stem Cell* 4, 170-179.

Kikuta,T., Shimazaki,C., Ashihara,E., Sudo,Y., Hirai,H., Sumikuma,T., Yamagata,N., Inaba,T., Fujita,N., Kina,T., and Nakagawa,M. (2000). Mobilization of hematopoietic primitive and committed progenitor cells into blood in mice by anti-vascular adhesion molecule-1 antibody alone or in combination with granulocyte colony-stimulating factor. *Exp. Hematol.* 28, 311-317.

Kim,C.H. and Broxmeyer,H.E. (1998). In vitro behavior of hematopoietic progenitor cells under the influence of chemoattractants: stromal cell-derived factor-1, steel factor, and the bone marrow environment. *Blood* 91, 100-110.

- Kim,N. and Luster,A.D. (2007). Regulation of immune cells by eicosanoid receptors. *ScientificWorldJournal*. 7, 1307-1328.
- Kincade,P.W., Lee,G., Fernandes,G., Moore,M.A., Williams,N., and Good,R.A. (1979). Abnormalities in clonable B lymphocytes and myeloid progenitors in autoimmune NZB mice. *Proc Natl Acad Sci U. S. A* 76, 3464-3468.
- King,A.G., Horowitz,D., Dillon,S.B., Levin,R., Farese,A.M., MacVittie,T.J., and Pelus,L.M. (2001). Rapid mobilization of murine hematopoietic stem cells with enhanced engraftment properties and evaluation of hematopoietic progenitor cell mobilization in rhesus monkeys by a single injection of SB-251353, a specific truncated form of the human CXC chemokine GRObeta. *Blood* 97, 1534-1542.
- Kirito,K., Hu,Y., and Komatsu,N. (2009). HIF-1 prevents the overproduction of mitochondrial ROS after cytokine stimulation through induction of PDK-1. *Cell Cycle* 8, 2844-2849.
- Kishimoto,S., Muramatsu,M., Gokoh,M., Oka,S., Waku,K., and Sugiura,T. (2005). Endogenous cannabinoid receptor ligand induces the migration of human natural killer cells. *J. Biochem. (Tokyo)* 137, 217-223.
- Klein,T.W., Lane,B., Newton,C.A., and Friedman,H. (2000). The cannabinoid system and cytokine network. *Proc. Soc. Exp. Biol. Med.* 225, 1-8.
- Klein,T.W., Newton,C., Larsen,K., Lu,L., Perkins,I., Nong,L., and Friedman,H. (2003). The cannabinoid system and immune modulation. *J. Leukoc. Biol.* 74, 486-496.
- Kollet,O., Spiegel,A., Peled,A., Petit,I., Byk,T., HersHKoviz,R., Guetta,E., Barkai,G., Nagler,A., and Lapidot,T. (2001). Rapid and efficient homing of human CD34(+)CD38(-/low)CXCR4(+) stem and progenitor cells to the bone marrow and spleen of NOD/SCID and NOD/SCID/B2m(null) mice. *Blood* 97, 3283-3291.
- Kollet,O., Dar,A., Shvitiel,S., Kalinkovich,A., Lapid,K., Sztainberg,Y., Tesio,M., Samstein,R.M., Goichberg,P., Spiegel,A., Elson,A., and Lapidot,T. (2006). Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. *Nat. Med.* 12, 657-664.
- Kondo,M., Weissman,I.L., and Akashi,K. (1997). Identification of clonogenic common lymphoid progenitors in mouse bone marrow. *Cell* 91, 661-672.
- Kondo,M., Wagers,A.J., Manz,M.G., Prohaska,S.S., Scherer,D.C., Beilhack,G.F., Shizuru,J.A., and Weissman,I.L. (2003). Biology of hematopoietic stem cells and progenitors: implications for clinical application. *Annu. Rev. Immunol.* 21, 759-806.

- Konger,R.L., Brouxhon,S., Partillo,S., VanBuskirk,J., and Pentland,A.P. (2005). The EP3 receptor stimulates ceramide and diacylglycerol release and inhibits growth of primary keratinocytes. *Exp. Dermatol.* 14, 914-922.
- Kopp,H.G., Ramos,C.A., and Rafii,S. (2006). Contribution of endothelial progenitors and proangiogenic hematopoietic cells to vascularization of tumor and ischemic tissue. *Curr. Opin. Hematol.* 13, 175-181.
- Koury,M.J. (1992). Programmed cell death (apoptosis) in hematopoiesis. *Exp. Hematol.* 20, 391-394.
- Kouvaris,J.R., Kouloulis,V.E., and Vlahos,L.J. (2007). Amifostine: the first selective-target and broad-spectrum radioprotector. *Oncologist.* 12, 738-747.
- Kozak,K.R., Crews,B.C., Ray,J.L., Tai,H.H., Morrow,J.D., and Marnett,L.J. (2001). Metabolism of prostaglandin glycerol esters and prostaglandin ethanolamides in vitro and in vivo. *J. Biol. Chem.* 276, 36993-36998.
- Kozak,K.R., Crews,B.C., Morrow,J.D., Wang,L.H., Ma,Y.H., Weinander,R., Jakobsson,P.J., and Marnett,L.J. (2002). Metabolism of the endocannabinoids, 2-arachidonylglycerol and anandamide, into prostaglandin, thromboxane, and prostacyclin glycerol esters and ethanolamides. *J. Biol. Chem.* 277, 44877-44885.
- Kozubik,A., Hofmanova,J., Pospisil,M., Netikova,J., Hola,J., and Lojek,A. (1994). Effects of drugs inhibiting prostaglandin or leukotriene biosynthesis on postirradiation haematopoiesis in mouse. *Int. J. Radiat. Biol.* 65, 369-377.
- Krause,D.S., Fackler,M.J., Civin,C.I., and May,W.S. (1996). CD34: structure, biology, and clinical utility. *Blood* 87, 1-13.
- Kretchmar,A.L. and Conover,W.R. (1970). A difference between spleen-derived and bone marrow-derived colony-forming units in ability to protect lethally irradiated mice. *Blood* 36, 772-776.
- Krishnamachary,B., Berg-Dixon,S., Kelly,B., Agani,F., Feldser,D., Ferreira,G., Iyer,N., LaRusch,J., Pak,B., Taghavi,P., and Semenza,G.L. (2003). Regulation of colon carcinoma cell invasion by hypoxia-inducible factor 1. *Cancer Res.* 63, 1138-1143.
- Kroger,N., Renges,H., Sonnenberg,S., Kruger,W., Gutensohn,K., Dielschneider,T., Cortes-Dericks,L., and Zander,A.R. (2002). Stem cell mobilisation with 16 microg/kg vs 10 microg/kg of G-CSF for allogeneic transplantation in healthy donors. *Bone Marrow Transplant.* 29, 727-730.

Krysan,K., Merchant,F.H., Zhu,L., Dohadwala,M., Luo,J., Lin,Y., Heuze-Vourc'h,N., Pold,M., Seligson,D., Chia,D., Goodglick,L., Wang,H., Strieter,R., Sharma,S., and Dubinett,S. (2004). COX-2-dependent stabilization of survivin in non-small cell lung cancer. *FASEB J.* 18, 206-208.

Kunikata,T., Araki,H., Takeeda,M., Kato,S., and Takeuchi,K. (2001). Prostaglandin E prevents indomethacin-induced gastric and intestinal damage through different EP receptor subtypes. *J. Physiol Paris* 95, 157-163.

Kurihara,R., Tohyama,Y., Matsusaka,S., Naruse,H., Kinoshita,E., Tsujioka,T., Katsumata,Y., and Yamamura,H. (2006). Effects of peripheral cannabinoid receptor ligands on motility and polarization in neutrophil-like HL60 cells and human neutrophils. *J. Biol. Chem.* 281, 12908-12918.

Kurland,J.I., Hadden,J.W., and Moore,M.A. (1977). Role of cyclic nucleotides in the proliferation of committed granulocyte-macrophage progenitor cells. *Cancer Res* 37, 4534-4538.

Kurland,J.I., Broxmeyer,H.E., Pelus,L.M., Bockman,R.S., and Moore,M.A. (1978). Role for monocyte-macrophage-derived colony-stimulating factor and prostaglandin E in the positive and negative feedback control of myeloid stem cell proliferation. *Blood* 52, 388-407.

Kurland,J.I., Pelus,L.M., Ralph,P., Bockman,R.S., and Moore,M.A. (1979). Induction of prostaglandin E synthesis in normal and neoplastic macrophages: role for colony-stimulating factor(s) distinct from effects on myeloid progenitor cell proliferation. *Proc. Natl. Acad. Sci. U. S. A* 76, 2326-2330.

Kurnick,J.E. and Robison,W.A. (1971). Colony growth of human peripheral white blood cells in vitro. *Blood* 37, 136-141.

Kurzrock,R. and Lieb,C.C. (1930). Biochemical studies of human semen. II. The action of semen on the human uterus. *Proc. Soc. Exp. Biol. Med.* 26, 268-272.

Lanzkron,S.M., Collector,M.I., and Sharkis,S.J. (1999). Homing of long-term and short-term engrafting cells in vivo. *Ann. N. Y. Acad. Sci.* 872, 48-54.

Lapidot,T., Pflumio,F., Doedens,M., Murdoch,B., Williams,D.E., and Dick,J.E. (1992). Cytokine stimulation of multilineage hematopoiesis from immature human cells engrafted in SCID mice. *Science* 255, 1137-1141.

Lapidot,T., Dar,A., and Kollet,O. (2005). How do stem cells find their way home? *Blood* 106, 1901-1910.

Lataillade,J.J., Clay,D., Dupuy,C., Rigal,S., Jasmin,C., Bourin,P., and Le Bousse-Kerdiles,M.C. (2000). Chemokine SDF-1 enhances circulating CD34(+) cell proliferation in synergy with cytokines: possible role in progenitor survival. *Blood* 95, 756-768.

Lazarus,M. (2006). The differential role of prostaglandin E2 receptors EP3 and EP4 in regulation of fever. *Mol. Nutr. Food Res.* 50, 451-455.

Lee,S.P., Serezani,C.H., Medeiros,A.I., Ballinger,M.N., and Peters-Golden,M. (2009). Crosstalk between prostaglandin E2 and leukotriene B4 regulates phagocytosis in alveolar macrophages via combinatorial effects on cyclic AMP. *J. Immunol.* 182, 530-537.

Lee,Y., Gotoh,A., Kwon,H.J., You,M., Kohli,L., Mantel,C., Cooper,S., Hangoc,G., Miyazawa,K., Ohyashiki,K., and Broxmeyer,H.E. (2002). Enhancement of intracellular signaling associated with hematopoietic progenitor cell survival in response to SDF-1/CXCL12 in synergy with other cytokines. *Blood* 99, 4307-4317.

Legler,D.F., Krause,P., Scandella,E., Singer,E., and Groettrup,M. (2006). Prostaglandin E2 is generally required for human dendritic cell migration and exerts its effect via EP2 and EP4 receptors. *J. Immunol.* 176, 966-973.

Leitner,S.P., Bosl,G.J., and Pelus,L.M. (1987). Abnormal colony formation and prostaglandin E responsiveness of myeloid progenitor cells in patients cured of germ cell neoplasms after combination chemotherapy. *Cancer* 60, 312-317.

Lemieux,M.E., Rebel,V.I., Lansdorp,P.M., and Eaves,C.J. (1995). Characterization and purification of a primitive hematopoietic cell type in adult mouse marrow capable of lymphomyeloid differentiation in long-term marrow "switch" cultures. *Blood* 86, 1339-1347.

Lemischka,I.R., Raulet,D.H., and Mulligan,R.C. (1986). Developmental potential and dynamic behavior of hematopoietic stem cells. *Cell* 45, 917-927.

Leon,M.B., Baim,D.S., Popma,J.J., Gordon,P.C., Cutlip,D.E., Ho,K.K., Giambartolomei,A., Diver,D.J., Lasorda,D.M., Williams,D.O., Pocock,S.J., and Kuntz,R.E. (1998). A clinical trial comparing three antithrombotic-drug regimens after coronary-artery stenting. Stent Anticoagulation Restenosis Study Investigators. *N. Engl. J. Med* 339, 1665-1671.

Leslie,C.C. (2004). Regulation of arachidonic acid availability for eicosanoid production. *Biochem. Cell Biol.* 82, 1-17.

- Leung,C.G., Xu,Y., Mularski,B., Liu,H., Gurbuxani,S., and Crispino,J.D. (2007). Requirements for survivin in terminal differentiation of erythroid cells and maintenance of hematopoietic stem and progenitor cells. *J Exp Med* 204, 1603-1611.
- Levesque,J.P., Leavesley,D.I., Niutta,S., Vadas,M., and Simmons,P.J. (1995). Cytokines increase human hemopoietic cell adhesiveness by activation of very late antigen (VLA)-4 and VLA-5 integrins. *J. Exp. Med.* 181, 1805-1815.
- Levesque,J.P., Takamatsu,Y., Nilsson,S.K., Haylock,D.N., and Simmons,P.J. (2001). Vascular cell adhesion molecule-1 (CD106) is cleaved by neutrophil proteases in the bone marrow following hematopoietic progenitor cell mobilization by granulocyte colony-stimulating factor. *Blood* 98, 1289-1297.
- Levesque,J.P., Winkler,I.G., Hendy,J., Williams,B., Helwani,F., Barbier,V., Nowlan,B., and Nilsson,S.K. (2007). Hematopoietic progenitor cell mobilization results in hypoxia with increased hypoxia-inducible transcription factor-1 alpha and vascular endothelial growth factor A in bone marrow. *Stem Cells* 25, 1954-1965.
- Levy,A.P., Levy,N.S., Wegner,S., and Goldberg,M.A. (1995). Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. *J. Biol. Chem.* 270, 13333-13340.
- Li,C.J., Chang,J.K., Chou,C.H., Wang,G.J., and Ho,M.L. (2010). The PI3K/Akt/FOXO3a/p27Kip1 signaling contributes to anti-inflammatory drug-suppressed proliferation of human osteoblasts. *Biochem. Pharmacol.* 79, 926-937.
- Li,W., Johnson,S.A., Shelley,W.C., and Yoder,M.C. (2004). Hematopoietic stem cell repopulating ability can be maintained in vitro by some primary endothelial cells. *Exp. Hematol.* 32, 1226-1237.
- Ligresti,A., Cascio,M.G., and Di,M., V (2005). Endocannabinoid metabolic pathways and enzymes. *Curr. Drug Targets. CNS. Neurol. Disord.* 4, 615-623.
- Liles,W.C., Broxmeyer,H.E., Rodger,E., Wood,B., Hubel,K., Cooper,S., Hangoc,G., Bridger,G.J., Henson,G.W., Calandra,G., and Dale,D.C. (2003). Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood* 102, 2728-2730.
- Liles,W.C., Rodger,E., Broxmeyer,H.E., Dehner,C., Badel,K., Calandra,G., Christensen,J., Wood,B., Price,T.H., and Dale,D.C. (2005). Augmented mobilization and collection of CD34+ hematopoietic cells from normal human volunteers stimulated with granulocyte-colony-stimulating factor by single-dose administration of AMD3100, a CXCR4 antagonist. *Transfusion* 45, 295-300.

- Lin,Q., Lee,Y.J., and Yun,Z. (2006). Differentiation arrest by hypoxia. *J. Biol. Chem.* 281, 30678-30683.
- Lindemann,A. and Rumberger,B. (1993). Vascular complications in patients treated with granulocyte colony-stimulating factor (G-CSF). *Eur. J. Cancer* 29A, 2338-2339.
- Lindgren,J.A., Stenke,L., Mansour,M., Edenius,C., Lauren,L., Nasman-Glaser,B., Ericsson,I., and Reizenstein,P. (1993). Formation and effects of leukotrienes and lipoxins in human bone marrow. *J. Lipid Mediat.* 6, 313-320.
- Liu,B., Buckley,S.M., Lewis,I.D., Goldman,A.I., Wagner,J.E., and van der Loo,J.C. (2003). Homing defect of cultured human hematopoietic cells in the NOD/SCID mouse is mediated by Fas/CD95. *Exp. Hematol.* 31, 824-832.
- Liu,X.H., Kirschenbaum,A., Lu,M., Yao,S., Dosoretz,A., Holland,J.F., and Levine,A.C. (2002). Prostaglandin E2 induces hypoxia-inducible factor-1alpha stabilization and nuclear localization in a human prostate cancer cell line. *J Biol Chem* 277, 50081-50086.
- Liu,Y., Hangoc,G., Campbell,T.B., Goodman,M., Tao,W., Pollok,K., Srour,E.F., and Broxmeyer,H.E. (2008). Identification of parameters required for efficient lentiviral vector transduction and engraftment of human cord blood CD34(+) NOD/SCID-repopulating cells. *Exp. Hematol.* 36, 947-956.
- Lo,C.C., Fleming,H.E., Wu,J.W., Zhao,C.X., Miake-Lye,S., Fujisaki,J., Cote,D., Rowe,D.W., Lin,C.P., and Scadden,D.T. (2009). Live-animal tracking of individual haematopoietic stem/progenitor cells in their niche. *Nature* 457, 92-96.
- Lombard,C., Nagarkatti,M., and Nagarkatti,P. (2007). CB2 cannabinoid receptor agonist, JWH-015, triggers apoptosis in immune cells: potential role for CB2-selective ligands as immunosuppressive agents. *Clin. Immunol.* 122, 259-270.
- Lord,B.I., Testa,N.G., and Hendry,J.H. (1975). The relative spatial distributions of CFUs and CFUc in the normal mouse femur. *Blood* 46, 65-72.
- Lorenz,E., Uphoff,D., REID,T.R., and Shelton,E. (1951). Modification of irradiation injury in mice and guinea pigs by bone marrow injections. *J. Natl. Cancer Inst.* 12, 197-201.
- Lorenz,M., Slaughter,H.S., Wescott,D.M., Carter,S.I., Schnyder,B., Dinchuk,J.E., and Car,B.D. (1999). Cyclooxygenase-2 is essential for normal recovery from 5-fluorouracil-induced myelotoxicity in mice. *Exp Hematol* 27, 1494-1502.

Lu,L., Pelus,L.M., and Broxmeyer,H.E. (1984). Modulation of the expression of HLA-DR (Ia) antigens and the proliferation of human erythroid (BFU-E) and multipotential (CFU-GEMM) progenitor cells by prostaglandin E. *Exp. Hematol.* 12, 741-748.

Lu,L., Pelus,L.M., Broxmeyer,H.E., Moore,M.A., Wachter,M., Walker,D., and Platzer,E. (1986). Enhancement of the proliferation of human marrow erythroid (BFU-E) progenitor cells by prostaglandin E requires the participation of OKT8-positive T lymphocytes and is associated with the density expression of major histocompatibility complex class II antigens on BFU-E. *Blood* 68, 126-133.

Lu,L., Pelus,L.M., Piacibello,W., Moore,M.A., Hu,W., and Broxmeyer,H.E. (1987). Prostaglandin E acts at two levels to enhance colony formation in vitro by erythroid (BFU-E) progenitor cells. *Exp. Hematol.* 15, 765-771.

Luczynski,W., Krawczuk-Rybak,M., and Stasiak-Barmuta,A. (2008). [Myeloid-derived suppressor cells - the new mechanism of immunosuppression in cancer]. *Postepy Hig. Med. Dosw. (Online.)* 62, 18-22.

MacVittie,T.J., Farese,A.M., and Jackson,W., III (2005). Defining the full therapeutic potential of recombinant growth factors in the post radiation-accident environment: the effect of supportive care plus administration of G-CSF. *Health Phys.* 89, 546-555.

Main,J.M. and Prehn,R.T. (1955). Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. *J. Natl. Cancer Inst.* 15, 1023-1029.

Malcher-Lopes,R., Franco,A., and Tasker,J.G. (2008). Glucocorticoids shift arachidonic acid metabolism toward endocannabinoid synthesis: a non-genomic anti-inflammatory switch. *Eur. J. Pharmacol.* 583, 322-339.

Massberg,S., Schaerli,P., Knezevic-Maramica,I., Kollnberger,M., Tubo,N., Moseman,E.A., Huff,I.V., Junt,T., Wagers,A.J., Mazo,I.B., and von Andrian,U.H. (2007). Immunosurveillance by hematopoietic progenitor cells trafficking through blood, lymph, and peripheral tissues. *Cell* 131, 994-1008.

Massi,P., Valenti,M., Vaccani,A., Gasperi,V., Perletti,G., Marras,E., Fezza,F., Maccarrone,M., and Parolaro,D. (2008). 5-Lipoxygenase and anandamide hydrolase (FAAH) mediate the antitumor activity of cannabidiol, a non-psychoactive cannabinoid. *J. Neurochem.* 104, 1091-1100.

Massoumi,R. and Sjolander,A. (2007). The role of leukotriene receptor signaling in inflammation and cancer. *ScientificWorldJournal.* 7, 1413-1421.

- Matsuzaki,Y., Kinjo,K., Mulligan,R.C., and Okano,H. (2004). Unexpectedly efficient homing capacity of purified murine hematopoietic stem cells. *Immunity*. 20, 87-93.
- Maubach,K.A., Davis,R.J., Clark,D.E., Fenton,G., Lockey,P.M., Clark,K.L., Oxford,A.W., Hagan,R.M., Routledge,C., and Coleman,R.A. (2009). BGC20-1531, a novel, potent and selective prostanoid EP receptor antagonist: a putative new treatment for migraine headache. *Br. J. Pharmacol.* 156, 316-327.
- McAllister,S.D. and Glass,M. (2002). CB(1) and CB(2) receptor-mediated signalling: a focus on endocannabinoids. *Prostaglandins Leukot. Essent. Fatty Acids* 66, 161-171.
- McCredie,K.B., Hersh,E.M., and Freireich,E.J. (1971). Cells capable of colony formation in the peripheral blood of man. *Science* 171, 293-294.
- McCune,J.M., Namikawa,R., Kaneshima,H., Shultz,L.D., Lieberman,M., and Weissman,I.L. (1988). The SCID-hu mouse: murine model for the analysis of human hematolymphoid differentiation and function. *Science* 241, 1632-1639.
- McKinney-Freeman,S. and Goodell,M.A. (2004). Circulating hematopoietic stem cells do not efficiently home to bone marrow during homeostasis. *Exp. Hematol.* 32, 868-876.
- McLeod,D.L., Shreeve,M.M., and Axelrad,A.A. (1974). Improved plasma culture system for production of erythrocytic colonies in vitro: quantitative assay method for CFU-E. *Blood* 44, 517-534.
- McLeod,D.L., Shreve,M.M., and Axelrad,A.A. (1976). Induction of megakaryocyte colonies with platelet formation in vitro. *Nature* 261, 492-494.
- McQuaker,I.G., Hunter,A.E., Pacey,S., Haynes,A.P., Iqbal,A., and Russell,N.H. (1997). Low-dose filgrastim significantly enhances neutrophil recovery following autologous peripheral-blood stem-cell transplantation in patients with lymphoproliferative disorders: evidence for clinical and economic benefit. *J. Clin. Oncol.* 15, 451-457.
- McQuibban,G.A., Butler,G.S., Gong,J.H., Bendall,L., Power,C., Clark-Lewis,I., and Overall,C.M. (2001). Matrix metalloproteinase activity inactivates the CXC chemokine stromal cell-derived factor-1. *J Biol Chem* 276, 43503-43508.
- Metcalf,D., Moore,M.A., and Warner,N.L. (1969). Colony formation in vitro by myelomonocytic leukemic cells. *J. Natl. Cancer Inst.* 43, 983-1001.

Metcalf,D. and Moore,M.A. (1970). Factors modifying stem cell proliferation of myelomonocytic leukemic cells in vitro and in vivo. *J. Natl. Cancer Inst.* **44**, 801-808.

Metcalf,D., MacDonald,H.R., Odartchenko,N., and Sordat,B. (1975a). Growth of mouse megakaryocyte colonies in vitro. *Proc. Natl. Acad. Sci. U. S. A* **72**, 1744-1748.

Metcalf,D., Warner,N.L., Nossal,G.J., Miller,J.F., Shortman,K., and Rabellino,E. (1975b). Growth of B lymphocyte colonies in vitro from mouse lymphoid organs. *Nature* **255**, 630-632.

Metcalf,D., Nossal,G.J., Warner,N.L., Miller,J.F., Mandel,T.E., Layton,J.E., and Gutman,G.A. (1975c). Growth of B-lymphocyte colonies in vitro. *J. Exp. Med.* **142**, 1534-1549.

Metcalf,D. and Nicola,N.A. (1983). Proliferative effects of purified granulocyte colony-stimulating factor (G-CSF) on normal mouse hemopoietic cells. *J. Cell Physiol* **116**, 198-206.

Miller,S.B. (2006). Prostaglandins in health and disease: an overview. *Semin. Arthritis Rheum.* **36**, 37-49.

Mitchell,J.A. and Warner,T.D. (2006). COX isoforms in the cardiovascular system: understanding the activities of non-steroidal anti-inflammatory drugs. *Nat. Rev. Drug Discov.* **5**, 75-86.

Mitchell,M.D., Sato,T.A., Wang,A., Keelan,J.A., Ponnampalam,A.P., and Glass,M. (2008). Cannabinoids stimulate prostaglandin production by human gestational tissues through a tissue- and CB1-receptor-specific mechanism. *Am. J. Physiol Endocrinol. Metab* **294**, E352-E356.

Miyaura,C., Inada,M., Matsumoto,C., Ohshiba,T., Uozumi,N., Shimizu,T., and Ito,A. (2003). An essential role of cytosolic phospholipase A2alpha in prostaglandin E2-mediated bone resorption associated with inflammation. *J Exp Med* **197**, 1303-1310.

Mohle,R., Bautz,F., Denzlinger,C., and Kanz,L. (2001). Transendothelial migration of hematopoietic progenitor cells. Role of chemotactic factors. *Ann. N. Y. Acad. Sci.* **938**, 26-34.

Mohty,M., Kuentz,M., Michallet,M., Bourhis,J.H., Milpied,N., Sutton,L., Jouet,J.P., Attal,M., Bordigoni,P., Cahn,J.Y., Boiron,J.M., and Blaise,D. (2002). Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study. *Blood* **100**, 3128-3134.

- Moore,A.H., Olschowka,J.A., Williams,J.P., Okunieff,P., and O'Banion,M.K. (2005). Regulation of prostaglandin E2 synthesis after brain irradiation. *Int J Radiat. Oncol Biol Phys.* 62, 267-272.
- Moore,M.A., Mertelsmann,R., and Pelus,L.M. (1981). Phenotypic evaluation of chronic myeloid leukemia. *Blood Cells* 7, 217-236.
- Morrison,S.J. and Weissman,I.L. (1994). The long-term repopulating subset of hematopoietic stem cells is deterministic and isolatable by phenotype. *Immunity.* 1, 661-673.
- Morrison,S.J. and Kimble,J. (2006). Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* 441, 1068-1074.
- Moulder,J.E. (2004). Post-irradiation approaches to treatment of radiation injuries in the context of radiological terrorism and radiation accidents: a review. *Int. J. Radiat. Biol.* 80, 3-10.
- Muller-Sieburg,C.E., Whitlock,C.A., and Weissman,I.L. (1986). Isolation of two early B lymphocyte progenitors from mouse marrow: a committed pre-pre-B cell and a clonogenic Thy-1-lo hematopoietic stem cell. *Cell* 44, 653-662.
- Muller-Sieburg,C.E., Cho,R.H., Thoman,M., Adkins,B., and Sieburg,H.B. (2002). Deterministic regulation of hematopoietic stem cell self-renewal and differentiation. *Blood* 100, 1302-1309.
- Muller-Sieburg,C.E., Cho,R.H., Karlsson,L., Huang,J.F., and Sieburg,H.B. (2004). Myeloid-biased hematopoietic stem cells have extensive self-renewal capacity but generate diminished lymphoid progeny with impaired IL-7 responsiveness. *Blood* 103, 4111-4118.
- Muller-Sieburg,C.E. and Sieburg,H.B. (2006a). Clonal diversity of the stem cell compartment. *Curr Opin Hematol* 13, 243-248.
- Muller-Sieburg,C.E. and Sieburg,H.B. (2006b). The GOD of hematopoietic stem cells: a clonal diversity model of the stem cell compartment. *Cell Cycle* 5, 394-398.
- Murakami,M. and Kudo,I. (2004). Recent advances in molecular biology and physiology of the prostaglandin E2-biosynthetic pathway. *Prog. Lipid Res.* 43, 3-35.
- Murakami,M. and Kudo,I. (2006). Prostaglandin E synthase: a novel drug target for inflammation and cancer. *Curr. Pharm. Des* 12, 943-954.

Murineddu,G., Lazzari,P., Ruiu,S., Sanna,A., Loriga,G., Manca,I., Falzoi,M., Dessi,C., Curzu,M.M., Chelucci,G., Pani,L., and Pinna,G.A. (2006). Tricyclic pyrazoles. 4. Synthesis and biological evaluation of analogues of the robust and selective CB2 cannabinoid ligand 1-(2',4'-dichlorophenyl)-6-methyl-N-piperidin-1-yl-1,4-dihydroindeno[1,2-c]pyrazole-3-carboxamide. *J Med Chem* **49**, 7502-7512.

Murray,J., Ward,C., O'Flaherty,J.T., Dransfield,I., Haslett,C., Chilvers,E.R., and Rossi,A.G. (2003). Role of leukotrienes in the regulation of human granulocyte behaviour: dissociation between agonist-induced activation and retardation of apoptosis. *Br. J. Pharmacol.* **139**, 388-398.

Murray,L., Chen,B., Galy,A., Chen,S., Tushinski,R., Uchida,N., Negrin,R., Tricot,G., Jagannath,S., Vesole,D., and . (1995). Enrichment of human hematopoietic stem cell activity in the CD34+Thy-1+Lin- subpopulation from mobilized peripheral blood. *Blood* **85**, 368-378.

Na,N.T., Traver,D., Weissman,I.L., and Akashi,K. (2002). Myeloerythroid-restricted progenitors are sufficient to confer radioprotection and provide the majority of day 8 CFU-S. *J. Clin. Invest* **109**, 1579-1585.

Nagasawa,T., Hirota,S., Tachibana,K., Takakura,N., Nishikawa,S., Kitamura,Y., Yoshida,N., Kikutani,H., and Kishimoto,T. (1996). Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* **382**, 635-638.

Nakamura,R., Auayporn,N., Smith,D.D., Palmer,J., Sun,J.Y., Schriber,J., Pullarkat,V., Parker,P., Rodriguez,R., Stein,A., Rosenthal,J., Wang,S., Karanas,C., Gaal,K., Senitzer,D., and Forman,S.J. (2008). Impact of graft cell dose on transplant outcomes following unrelated donor allogeneic peripheral blood stem cell transplantation: higher CD34+ cell doses are associated with decreased relapse rates. *Biol. Blood Marrow Transplant.* **14**, 449-457.

Nakamura,Y., Ando,K., Chargui,J., Kawada,H., Sato,T., Tsuji,T., Hotta,T., and Kato,S. (1999). Ex vivo generation of CD34(+) cells from CD34(-) hematopoietic cells. *Blood* **94**, 4053-4059.

Nakeff,A. and Daniels-McQueen,S. (1976). In vitro colony assay for a new class of megakaryocyte precursor: colony-forming unit megakaryocyte (CFU-M). *Proc. Soc. Exp. Biol. Med.* **151**, 587-590.

Namba,T., Sugimoto,Y., Negishi,M., Irie,A., Ushikubi,F., Kakizuka,A., Ito,S., Ichikawa,A., and Narumiya,S. (1993). Alternative splicing of C-terminal tail of prostaglandin E receptor subtype EP3 determines G-protein specificity. *Nature* **365**, 166-170.

Narumiya,S. (2003). Prostanoids in immunity: roles revealed by mice deficient in their receptors. *Life Sci.* **74**, 391-395.

Negrin,R.S., Atkinson,K., Leemhuis,T., Hanania,E., Juttner,C., Tierney,K., Hu,W.W., Johnston,L.J., Shizurn,J.A., Stockerl-Goldstein,K.E., Blume,K.G., Weissman,I.L., Bower,S., Baynes,R., Dansey,R., Karanes,C., Peters,W., and Klein,J. (2000). Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with metastatic breast cancer. *Biol. Blood Marrow Transplant.* **6**, 262-271.

Negrotto,S., Pacienza,N., D'Atri,L.P., Pozner,R.G., Malaver,E., Torres,O., Lazzari,M.A., Gomez,R.M., and Schattner,M. (2006). Activation of cyclic AMP pathway prevents CD34(+) cell apoptosis. *Exp. Hematol.* **34**, 1420-1428.

Nemunaitis,J., Rosenfeld,C.S., Ash,R., Freedman,M.H., Deeg,H.J., Appelbaum,F., Singer,J.W., Flomenberg,N., Dalton,W., Elfenbein,G.J., and . (1995). Phase III randomized, double-blind placebo-controlled trial of rhGM-CSF following allogeneic bone marrow transplantation. *Bone Marrow Transplant.* **15**, 949-954.

Nibley,W.E. and Spangrude,G.J. (1998). Primitive stem cells alone mediate rapid marrow recovery and multilineage engraftment after transplantation. *Bone Marrow Transplant.* **21**, 345-354.

Nilsson,O., Fowler,C.J., and Jacobsson,S.O. (2006). The cannabinoid agonist WIN 55,212-2 inhibits TNF-alpha-induced neutrophil transmigration across ECV304 cells. *Eur. J. Pharmacol.* **547**, 165-173.

Nilsson,S.K., Johnston,H.M., and Coverdale,J.A. (2001). Spatial localization of transplanted hemopoietic stem cells: inferences for the localization of stem cell niches. *Blood* **97**, 2293-2299.

Nilsson,S.K., Johnston,H.M., Whitty,G.A., Williams,B., Webb,R.J., Denhardt,D.T., Bertoncello,I., Bendall,L.J., Simmons,P.J., and Haylock,D.N. (2005). Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. *Blood* **106**, 1232-1239.

Nirodi,C.S., Crews,B.C., Kozak,K.R., Morrow,J.D., and Marnett,L.J. (2004). The glyceryl ester of prostaglandin E2 mobilizes calcium and activates signal transduction in RAW264.7 cells. *Proc. Natl. Acad. Sci. U. S. A* **101**, 1840-1845.

Nishigaki,N., Negishi,M., and Ichikawa,A. (1996). Two Gs-coupled prostaglandin E receptor subtypes, EP2 and EP4, differ in desensitization and sensitivity to the metabolic inactivation of the agonist. *Mol. Pharmacol.* **50**, 1031-1037.

- Nishiguchi,I., Furuta,Y., Hunter,N., Murray,D., and Milas,L. (1990). Radioprotection of hematopoietic tissues in mice by indomethacin. *Radiat. Res.* 122, 188-192.
- Nocka,K.H., Ottman,O.G., and Pelus,L.M. (1989). The role of marrow accessory cell populations in the augmentation of human erythroid progenitor cell (BFU-E) proliferation by prostaglandin E. *Leuk. Res.* 13, 527-534.
- North,T.E., Goessling,W., Walkley,C.R., Lengerke,C., Kopani,K.R., Lord,A.M., Weber,G.J., Bowman,T.V., Jang,I.H., Grosser,T., Fitzgerald,G.A., Daley,G.Q., Orkin,S.H., and Zon,L.I. (2007). Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature* 447, 1007-1011.
- Nowell,P.C., COLE,L.J., Habermeyer,J.G., and Roan,P.L. (1956). Growth and continued function of rat marrow cells in x-radiated mice. *Cancer Res.* 16, 258-261.
- Nunia,V., Sancheti,G., and Goyal,P.K. (2007). Protection of Swiss albino mice against whole-body gamma irradiation by diltiazem. *Br. J. Radiol.* 80, 77-84.
- Ogawa,M., Porter,P.N., and Nakahata,T. (1983). Renewal and commitment to differentiation of hemopoietic stem cells (an interpretive review). *Blood* 61, 823-829.
- Ogawa,M., Matsuzaki,Y., Nishikawa,S., Hayashi,S., Kunisada,T., Sudo,T., Kina,T., Nakauchi,H., and Nishikawa,S. (1991). Expression and function of c-kit in hemopoietic progenitor cells. *J. Exp. Med.* 174, 63-71.
- Ogawa,M. (1993). Differentiation and proliferation of hematopoietic stem cells. *Blood* 81, 2844-2853.
- Ogden,D.A. and Mickliem,H.S. (1976). The fate of serially transplanted bone marrow cell populations from young and old donors. *Transplantation* 22, 287-293.
- Ohneda,O., Fennie,C., Zheng,Z., Donahue,C., La,H., Villacorta,R., Cairns,B., and Lasky,L.A. (1998). Hematopoietic stem cell maintenance and differentiation are supported by embryonic aorta-gonad-mesonephros region-derived endothelium. *Blood* 92, 908-919.
- Okada,S., Nakauchi,H., Nagayoshi,K., Nishikawa,S., Miura,Y., and Suda,T. (1992). In vivo and in vitro stem cell function of c-kit- and Sca-1-positive murine hematopoietic cells. *Blood* 80, 3044-3050.
- Okamoto,Y., Wang,J., Morishita,J., and Ueda,N. (2007). Biosynthetic pathways of the endocannabinoid anandamide. *Chem. Biodivers.* 4, 1842-1857.

Orlic,D., Kajstura,J., Chimenti,S., Limana,F., Jakoniuk,I., Quaini,F., Nadal-Ginard,B., Bodine,D.M., Leri,A., and Anversa,P. (2001a). Mobilized bone marrow cells repair the infarcted heart, improving function and survival. *Proc. Natl. Acad. Sci. U. S. A* 98, 10344-10349.

Orlic,D., Kajstura,J., Chimenti,S., Jakoniuk,I., Anderson,S.M., Li,B., Pickel,J., McKay,R., Nadal-Ginard,B., Bodine,D.M., Leri,A., and Anversa,P. (2001b). Bone marrow cells regenerate infarcted myocardium. *Nature* 410, 701-705.

Orschell-Traycoff,C.M., Hiatt,K., Dagher,R.N., Rice,S., Yoder,M.C., and Srour,E.F. (2000). Homing and engraftment potential of Sca-1(+)lin(-) cells fractionated on the basis of adhesion molecule expression and position in cell cycle. *Blood* 96, 1380-1387.

Osawa,M., Hanada,K., Hamada,H., and Nakauchi,H. (1996). Long-term lymphohematopoietic reconstitution by a single CD34-low/negative hematopoietic stem cell. *Science* 273, 242-245.

Panse,J.P., Heimfeld,S., Guthrie,K.A., Maris,M.B., Maloney,D.G., Baril,B.B., Little,M.T., Chauncey,T.R., Storer,B.E., Storb,R., and Sandmaier,B.M. (2005). Allogeneic peripheral blood stem cell graft composition affects early T-cell chimaerism and later clinical outcomes after non-myeloablative conditioning. *Br. J. Haematol.* 128, 659-667.

Panzer,U. and Uguccioni,M. (2004). Prostaglandin E2 modulates the functional responsiveness of human monocytes to chemokines. *Eur. J. Immunol.* 34, 3682-3689.

Papayannopoulou,T., Priestley,G.V., Nakamoto,B., Zafiropoulos,V., and Scott,L.M. (2001). Molecular pathways in bone marrow homing: dominant role of alpha(4)beta(1) over beta(2)-integrins and selectins. *Blood* 98, 2403-2411.

Papayannopoulou,T. (2004). Current mechanistic scenarios in hematopoietic stem/progenitor cell mobilization. *Blood* 103, 1580-1585.

Park,J.S., Jang,A.S., Park,S.W., Lee,Y.M., Uh,S.T., Kim,Y.H., Cha,J.Y., Park,S.M., and Park,C.S. (2010). Protection of leukotriene receptor antagonist against aspirin-induced bronchospasm in asthmatics. *Allergy Asthma Immunol. Res.* 2, 48-54.

Park,J.Y., Pillinger,M.H., and Abramson,S.B. (2006). Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases. *Clin. Immunol.* 119, 229-240.

Parmiani,G., Castelli,C., Pilla,L., Santinami,M., Colombo,M.P., and Rivoltini,L. (2007). Opposite immune functions of GM-CSF administered as vaccine adjuvant in cancer patients. *Ann. Oncol.* 18, 226-232.

Patinkin,D., Milman,G., Breuer,A., Fride,E., and Mechoulam,R. (2008). Endocannabinoids as positive or negative factors in hematopoietic cell migration and differentiation. *Eur. J. Pharmacol.* 595, 1-6.

Peled,A., Petit,I., Kollet,O., Magid,M., Ponomaryov,T., Byk,T., Nagler,A., Ben-Hur,H., Many,A., Shultz,L., Lider,O., Alon,R., Zipori,D., and Lapidot,T. (1999). Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* 283, 845-848.

Peled,A., Kollet,O., Ponomaryov,T., Petit,I., Franitza,S., Grabovsky,V., Slav,M.M., Nagler,A., Lider,O., Alon,R., Zipori,D., and Lapidot,T. (2000). The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. *Blood* 95, 3289-3296.

Pellmar,T.C. and Rockwell,S. (2005). Priority list of research areas for radiological nuclear threat countermeasures. *Radiat. Res.* 163, 115-123.

Pelus,L.M., Broxmeyer,H.E., Kurland,J.I., and Moore,M.A. (1979). Regulation of macrophage and granulocyte proliferation. Specificities of prostaglandin E and lactoferrin. *J. Exp. Med.* 150, 277-292.

Pelus,L.M., Broxmeyer,H.E., Clarkson,B.D., and Moore,M.A. (1980). Abnormal responsiveness of granulocyte-macrophage committed colony-forming cells from patients with chronic myeloid leukemia to inhibition by prostaglandin E1. *Cancer Res.* 40, 2512-2515.

Pelus,L.M., Broxmeyer,H.E., and Moore,M.A. (1981). Regulation of human myelopoiesis by prostaglandin E and lactoferrin. *Cell Tissue Kinet.* 14, 515-526.

Pelus,L.M. (1982). Association between colony forming units-granulocyte macrophage expression of Ia-like (HLA-DR) antigen and control of granulocyte and macrophage production. A new role for prostaglandin E. *J. Clin. Invest* 70, 568-578.

Pelus,L.M., Gold,E., Saletan,S., and Coleman,M. (1983). Restoration of responsiveness of chronic myeloid leukemia granulocyte-macrophage colony-forming cells to growth regulation in vitro following preincubation with prostaglandin E. *Blood* 62, 158-165.

Pelus,L.M. (1984). Prostaglandin-E-mediated modulation of human marrow CFU-GM Ia-antigen expression: kinetics and specificity. *Exp. Hematol.* 12, 831-837.

Pelus,L.M. and Vadhan-Raj,S. (1988). Modulation of responsiveness of chronic myelogenous leukemia granulocyte-macrophage colony-forming cells to growth regulation following in vivo treatment with recombinant gamma-interferon. *Am J Hematol* 28, 21-26.

Pelus,L.M. and Gentile,P.S. (1988). In vivo modulation of myelopoiesis by prostaglandin E2. III. Induction of suppressor cells in marrow and spleen capable of mediating inhibition of CFU-GM proliferation. *Blood* 71, 1633-1640.

Pelus,L.M., Ottmann,O.G., and Nocka,K.H. (1988). Synergistic inhibition of human marrow granulocyte-macrophage progenitor cells by prostaglandin E and recombinant interferon-alpha, -beta, and -gamma and an effect mediated by tumor necrosis factor. *J. Immunol.* 140, 479-484.

Pelus,L.M. (1989a). Blockade of prostaglandin biosynthesis in intact mice dramatically augments the expansion of committed myeloid progenitor cells (colony-forming units-granulocyte, macrophage) after acute administration of recombinant human IL-1 alpha. *J. Immunol.* 143, 4171-4179.

Pelus,L.M. (1989b). Modulation of myelopoiesis by prostaglandin E2: demonstration of a novel mechanism of action in vivo. *Immunol. Res.* 8, 176-184.

Pelus,L.M., Bian,H., King,A.G., and Fukuda,S. (2004). Neutrophil-derived MMP-9 mediates synergistic mobilization of hematopoietic stem and progenitor cells by the combination of G-CSF and the chemokines GRObeta/CXCL2 and GRObetaT/CXCL2delta4. *Blood* 103, 110-119.

Pelus,L.M., Bian,H., Fukuda,S., Wong,D., Merzouk,A., and Salari,H. (2005). The CXCR4 agonist peptide, CTCE-0021, rapidly mobilizes polymorphonuclear neutrophils and hematopoietic progenitor cells into peripheral blood and synergizes with granulocyte colony-stimulating factor. *Exp Hematol* 33, 295-307.

Pelus, L. M., Fukuda, S., and Bridger, G. The CXCR4 antagonist AMD3100 and the CXCR2 agonist GRO synergistically mobilize hematopoietic cells (HSC) with short and long term repopulating activity. *Blood* 108, 339. 2006a.

Pelus, L. M., Clapp, D. W., and Bridger, G. Suprasynergistic peripheral blood stem cell mobilization in normal and Fanconi Anemia knockout mice by the combination of G-CSF plus the CXCR4 antagonist AMD3100 and the CXCR2 agonist GROβ. *Blood* 108, 3185. 2006b.

Pelus, L. M. and Singh, P. The combination of AMD3100 plus GROβ rapidly mobilizes hematopoietic stem cells with enhanced homing, adhesion and survival properties. *Blood* 112, 71. 2008.

- Peng,X.H., Karna,P., Cao,Z., Jiang,B.H., Zhou,M., and Yang,L. (2006). Cross-talk between epidermal growth factor receptor and hypoxia-inducible factor-1alpha signal pathways increases resistance to apoptosis by up-regulating survivin gene expression. *J. Biol. Chem.* **281**, 25903-25914.
- Pertwee,R.G. (1999). Pharmacology of cannabinoid receptor ligands. *Curr Med Chem* **6**, 635-664.
- Pertwee,R.G. (2005). Inverse agonism and neutral antagonism at cannabinoid CB1 receptors. *Life Sci.* **76**, 1307-1324.
- Pertwee,R.G. (2006). Cannabinoid pharmacology: the first 66 years. *Br. J. Pharmacol.* **147 Suppl 1**, S163-S171.
- Phillips,R.J., Mestas,J., Gharaee-Kermani,M., Burdick,M.D., Sica,A., Belperio,J.A., Keane,M.P., and Strieter,R.M. (2005). Epidermal growth factor and hypoxia-induced expression of CXC chemokine receptor 4 on non-small cell lung cancer cells is regulated by the phosphatidylinositol 3-kinase/PTEN/AKT/mammalian target of rapamycin signaling pathway and activation of hypoxia inducible factor-1alpha. *J. Biol. Chem.* **280**, 22473-22481.
- Piacibello,W., Bruno,S., Sanavio,F., Droetto,S., Gunetti,M., Ailles,L., Santoni de,S.F., Viale,A., Gammaitoni,L., Lombardo,A., Naldini,L., and Aglietta,M. (2002). Lentiviral gene transfer and ex vivo expansion of human primitive stem cells capable of primary, secondary, and tertiary multilineage repopulation in NOD/SCID mice. Nonobese diabetic/severe combined immunodeficient. *Blood* **100**, 4391-4400.
- Piccoli,C., D'Aprile,A., Ripoli,M., Scrima,R., Boffoli,D., Tabilio,A., and Capitanio,N. (2007). The hypoxia-inducible factor is stabilized in circulating hematopoietic stem cells under normoxic conditions. *FEBS Lett* **581**, 3111-3119.
- Pizzo,P.A. (1984). Granulocytopenia and cancer therapy. Past problems, current solutions, future challenges. *Cancer* **54**, 2649-2661.
- Placzek,E.A., Okamoto,Y., Ueda,N., and Barker,E.L. (2008). Mechanisms for recycling and biosynthesis of endogenous cannabinoids anandamide and 2-arachidonylglycerol. *J. Neurochem.* **107**, 987-1000.
- Platzbecker,U., Prange-Krex,G., Bornhauser,M., Koch,R., Soucek,S., Aikele,P., Haack,A., Haag,C., Schuler,U., Berndt,A., Rutt,C., Ehninger,G., and Holig,K. (2001). Spleen enlargement in healthy donors during G-CSF mobilization of PBPCs. *Transfusion* **41**, 184-189.

Ploemacher,R.E., van der Sluijs,J.P., Voerman,J.S., and Brons,N.H. (1989). An in vitro limiting-dilution assay of long-term repopulating hematopoietic stem cells in the mouse. *Blood* 74, 2755-2763.

Ploemacher,R.E., van der Sluijs,J.P., van Beurden,C.A., Baert,M.R., and Chan,P.L. (1991). Use of limiting-dilution type long-term marrow cultures in frequency analysis of marrow-repopulating and spleen colony-forming hematopoietic stem cells in the mouse. *Blood* 78, 2527-2533.

Ploemacher,R.E., van der Loo,J.C., van Beurden,C.A., and Baert,M.R. (1993). Wheat germ agglutinin affinity of murine hemopoietic stem cell subpopulations is an inverse function of their long-term repopulating ability in vitro and in vivo. *Leukemia* 7, 120-130.

Pluznik,D.H. and Sachs,L. (1966). The induction of clones of normal mast cells by a substance from conditioned medium. *Exp. Cell Res.* 43, 553-563.

Ponomaryov,T., Peled,A., Petit,I., Taichman,R.S., Habler,L., Sandbank,J., Arenzana-Seisdedos,F., Magerus,A., Caruz,A., Fujii,N., Nagler,A., Lahav,M., Szyper-Kravitz,M., Zipori,D., and Lapidot,T. (2000). Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. *J. Clin. Invest* 106, 1331-1339.

Porecha,N.K., English,K., Hangoc,G., Broxmeyer,H.E., and Christopherson,K.W. (2006). Enhanced functional response to CXCL12/SDF-1 through retroviral overexpression of CXCR4 on M07e cells: implications for hematopoietic stem cell transplantation. *Stem Cells Dev.* 15, 325-333.

Pospisil,M., Netikova,J., Kozubik,A., and Pipalova,I. (1989). Effect of indomethacin, diclofenac sodium and sodium salicylate on peripheral blood cell counts in sublethally gamma-irradiated mice. *Strahlenther. Onkol.* 165, 627-631.

Poston,J.W., Sr. (2005). Warren K. Sinclair Keynote Address: Current challenges in countering radiological terrorism. *Health Phys.* 89, 450-456.

Powell,T.M., Paul,J.D., Hill,J.M., Thompson,M., Benjamin,M., Rodrigo,M., McCoy,J.P., Read,E.J., Khuu,H.M., Leitman,S.F., Finkel,T., and Cannon,R.O., III (2005). Granulocyte colony-stimulating factor mobilizes functional endothelial progenitor cells in patients with coronary artery disease. *Arterioscler. Thromb. Vasc. Biol.* 25, 296-301.

Powles,T., Te,P.R., Shamash,J., Chaplin,T., Propper,D., Joel,S., Oliver,T., and Liu,W.M. (2005). Cannabis-induced cytotoxicity in leukemic cell lines: the role of the cannabinoid receptors and the MAPK pathway. *Blood* 105, 1214-1221.

Prchal,J.F., Adamson,J.W., Steinmann,L., and Fialkow,P.J. (1976). Human erythroid colony formation in vitro: evidence for clonal origin. *J. Cell Physiol* 89, 489-492.

Pulsipher,M.A., Chitphakdithai,P., Logan,B.R., Leitman,S.F., Anderlini,P., Klein,J.P., Horowitz,M.M., Miller,J.P., King,R.J., and Confer,D.L. (2009). Donor, recipient, and transplant characteristics as risk factors after unrelated donor PBSC transplantation: beneficial effects of higher CD34+ cell dose. *Blood* 114, 2606-2616.

Purdie,J.W., Inhaber,E.R., Schneider,H., and Labelle,J.L. (1983). Interaction of cultured mammalian cells with WR-2721 and its thiol, WR-1065: implications for mechanisms of radioprotection. *Int. J. Radiat. Biol. Relat Stud. Phys. Chem. Med* 43, 517-527.

Purton,L.E. and Scadden,D.T. (2007). Limiting factors in murine hematopoietic stem cell assays. *Cell Stem Cell* 1, 263-270.

Qian,H., Tryggvason,K., Jacobsen,S.E., and Ekblom,M. (2006). Contribution of alpha6 integrins to hematopoietic stem and progenitor cell homing to bone marrow and collaboration with alpha4 integrins. *Blood* 107, 3503-3510.

Qian,H., Georges-Labouesse,E., Nystrom,A., Domogatskaya,A., Tryggvason,K., Jacobsen,S.E., and Ekblom,M. (2007). Distinct roles of integrins alpha6 and alpha4 in homing of fetal liver hematopoietic stem and progenitor cells. *Blood* 110, 2399-2407.

Rabbani,Z.N., Anscher,M.S., Folz,R.J., Archer,E., Huang,H., Chen,L., Golson,M.L., Samulski,T.S., Dewhirst,M.W., and Vujaskovic,Z. (2005). Overexpression of extracellular superoxide dismutase reduces acute radiation induced lung toxicity. *BMC. Cancer* 5, 59.

Rafii,S. and Lyden,D. (2003). Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat. Med* 9, 702-712.

Raisz,L.G., Vanderhoek,J.Y., Simmons,H.A., Kream,B.E., and Nicolaou,K.C. (1979). Prostaglandin synthesis by fetal rat bone in vitro: evidence for a role of prostacyclin. *Prostaglandins* 17, 905-914.

Ramirez,M., Segovia,J.C., Benet,I., Arbona,C., Guenechea,G., Blaya,C., Garcia-Conde,J., Bueren,J.A., and Prosper,F. (2001). Ex vivo expansion of umbilical cord blood (UCB) CD34(+) cells alters the expression and function of alpha 4 beta 1 and alpha 5 beta 1 integrins. *Br. J. Haematol.* 115, 213-221.

- Ramirez,P., Rettig,M.P., Uy,G.L., Deych,E., Holt,M.S., Ritchey,J.K., and DiPersio,J.F. (2009). BIO5192, a small molecule inhibitor of VLA-4, mobilizes hematopoietic stem and progenitor cells. *Blood* 114, 1340-1343.
- Ramshaw,H.S., Crittenden,R.B., Dooner,M., Peters,S.O., Rao,S.S., and Quesenberry,P.J. (1995). High levels of engraftment with a single infusion of bone marrow cells into normal unprepared mice. *Biol. Blood Marrow Transplant.* 1, 74-80.
- Rayman,N., Lam,K.H., Van,L.J., Mulder,A.H., Budel,L.M., Lowenberg,B., Sonneveld,P., and Delwel,R. (2007). The expression of the peripheral cannabinoid receptor on cells of the immune system and non-Hodgkin's lymphomas. *Leuk. Lymphoma* 48, 1389-1399.
- Rebel,V.I., Tanaka,M., Lee,J.S., Hartnett,S., Pulsipher,M., Nathan,D.G., Mulligan,R.C., and Sieff,C.A. (1999). One-day ex vivo culture allows effective gene transfer into human nonobese diabetic/severe combined immune-deficient repopulating cells using high-titer vesicular stomatitis virus G protein pseudotyped retrovirus. *Blood* 93, 2217-2224.
- Regan,J.W. (2003). EP2 and EP4 prostanoid receptor signaling. *Life Sci.* 74, 143-153.
- Reisner,Y. and Martelli,M.F. (2000). Transplantation tolerance induced by "mega dose" CD34+ cell transplants. *Exp Hematol* 28, 119-127.
- Reno,F., Baj,G., Surico,N., and Cannas,M. (2004). Exogenous prostaglandin E2 inhibits TPA induced matrix metalloproteinase-9 production in MCF-7 cells. *Prostaglandins Other Lipid Mediat.* 73, 237-247.
- Rettig, M. P., McFarland, K., Ritchey, J., Holt, M., Deych, E., Lopez, S., Gabriel, J., Bauer, S., Cashen, A., and DiPersio, J. F. Preferential Mobilization of CD34+ Plasmacytoid Dendritic Cell Precursors by Plerixafor. *Blood* 114[32]. 2009.
- Ricciardi,A., Elia,A.R., Cappello,P., Puppo,M., Vanni,C., Fardin,P., Eva,A., Munroe,D., Wu,X., Giovarelli,M., and Varesio,L. (2008). Transcriptome of hypoxic immature dendritic cells: modulation of chemokine/receptor expression. *Mol. Cancer Res.* 6, 175-185.
- Rinder,H.M., Tracey,J.B., Souhrada,M., Wang,C., Gagnier,R.P., and Wood,C.C. (2002). Effects of meloxicam on platelet function in healthy adults: a randomized, double-blind, placebo-controlled trial. *J. Clin. Pharmacol.* 42, 881-886.

- Ringden,O., Remberger,M., Runde,V., Bornhauser,M., Blau,I.W., Basara,N., Holig,K., Beelen,D.W., Hagglund,H., Basu,O., Ehninger,G., and Fauser,A.A. (2000). Faster engraftment of neutrophils and platelets with peripheral blood stem cells from unrelated donors: a comparison with marrow transplantation. *Bone Marrow Transplant.* 25 *Suppl* 2, S6-S8.
- Rosch,S., Ramer,R., Brune,K., and Hinz,B. (2006). R(+)-methanandamide and other cannabinoids induce the expression of cyclooxygenase-2 and matrix metalloproteinases in human nonpigmented ciliary epithelial cells. *J. Pharmacol. Exp. Ther.* 316, 1219-1228.
- Rosendaal,M., Hodgson,G.S., and Bradley,T.R. (1979). Organization of haemopoietic stem cells: the generation-age hypothesis. *Cell Tissue Kinet.* 12, 17-29.
- Rossi,G.B., Migliaccio,A.R., Migliaccio,G., Lettieri,F., Di,R.M., Peschle,C., and Mastroberardino,G. (1980). In vitro interactions of PGE and cAMP with murine and human erythroid precursors. *Blood* 56, 74-79.
- Rowley,S.D., Donaldson,G., Lilleby,K., Bensinger,W.I., and Appelbaum,F.R. (2001). Experiences of donors enrolled in a randomized study of allogeneic bone marrow or peripheral blood stem cell transplantation. *Blood* 97, 2541-2548.
- Sackstein,R. (2004). The bone marrow is akin to skin: HCELL and the biology of hematopoietic stem cell homing. *J. Invest Dermatol.* 122, 1061-1069.
- Salcedo,R., Zhang,X., Young,H.A., Michael,N., Wasserman,K., Ma,W.H., Martins-Green,M., Murphy,W.J., and Oppenheim,J.J. (2003). Angiogenic effects of prostaglandin E2 are mediated by up-regulation of CXCR4 on human microvascular endothelial cells. *Blood* 102, 1966-1977.
- Salnikow,K., Donald,S.P., Bruick,R.K., Zhitkovich,A., Phang,J.M., and Kasprzak,K.S. (2004). Depletion of intracellular ascorbate by the carcinogenic metals nickel and cobalt results in the induction of hypoxic stress. *J. Biol. Chem.* 279, 40337-40344.
- Samuelsson,B., Morgenstern,R., and Jakobsson,P.J. (2007). Membrane prostaglandin E synthase-1: a novel therapeutic target. *Pharmacol. Rev.* 59, 207-224.
- Sandhya,T., Lathika,K.M., Pandey,B.N., Bhilwade,H.N., Chaubey,R.C., Priyadarsini,K.I., and Mishra,K.P. (2006). Protection against radiation oxidative damage in mice by Triphala. *Mutat. Res.* 609, 17-25.

- Sang,N., Zhang,J., and Chen,C. (2007). COX-2 oxidative metabolite of endocannabinoid 2-AG enhances excitatory glutamatergic synaptic transmission and induces neurotoxicity. *J. Neurochem.* 102, 1966-1977.
- Santoni de Sio,F. and Naldini,L. (2008). Short-term culture of human CD34+ cells for lentiviral gene transfer. In *Genetic Modification of Hematopoietic Stem Cells*, C.Baum, ed. (Totawa: Human Press), pp. 59-70.
- Schmits,R., Filmus,J., Gerwin,N., Senaldi,G., Kiefer,F., Kundig,T., Wakeham,A., Shahinian,A., Catzavelos,C., Rak,J., Furlonger,C., Zakarian,A., Simard,J.J., Ohashi,P.S., Paige,C.J., Gutierrez-Ramos,J.C., and Mak,T.W. (1997). CD44 regulates hematopoietic progenitor distribution, granuloma formation, and tumorigenicity. *Blood* 90, 2217-2233.
- Schmitt,T.M. and Zuniga-Pflucker,J.C. (2002). Induction of T cell development from hematopoietic progenitor cells by delta-like-1 in vitro. *Immunity.* 17, 749-756.
- Schmitz,N., Dreger,P., Suttorp,M., Rohwedder,E.B., Haferlach,T., Loffler,H., Hunter,A., and Russell,N.H. (1995). Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor). *Blood* 85, 1666-1672.
- Schofield,R. (1978). The relationship between the spleen colony-forming cell and the haemopoietic stem cell. *Blood Cells* 4, 7-25.
- Semenza,G.L., Neifelt,M.K., Chi,S.M., and Antonarakis,S.E. (1991). Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. *Proc. Natl. Acad. Sci. U. S. A* 88, 5680-5684.
- Senn,J.S., McCulloch,E.A., and Till,J.E. (1967). Comparison of colony-forming ability of normal and leukaemic human marrow in cell culture. *Lancet* 2, 597-598.
- Serhan,C.N. and Sheppard,K.A. (1990). Lipoxin formation during human neutrophil-platelet interactions. Evidence for the transformation of leukotriene A4 by platelet 12-lipoxygenase in vitro. *J. Clin. Invest* 85, 772-780.
- Serhan,C.N. and Levy,B. (2003). Novel pathways and endogenous mediators in anti-inflammation and resolution. *Chem. Immunol. Allergy* 83, 115-145.
- Serushago,B.A., Tanaka,K., Koga,Y., Taniguchi,K., and Nomoto,K. (1987). Positive effects of indomethacin on restoration of splenic nucleated cell populations in mice given sublethal irradiation. *Immunopharmacology* 14, 21-26.

Shaheen,M. and Broxmeyer,H.E. (2009). The humoral regulation of hematopoiesis. In *Hematology: Basic Principles and Practice*, R.Hoffman, E.J.J.Benz, S.J.Shattil, B.Furie, L.E.Silberstein, P.McGlave, H.Heslop, and J.Anastasi, eds. (Philadelphia: Elsevier Churchill Livingston), pp. 253-275.

Shamir,D., Keila,S., and Weinreb,M. (2004). A selective EP4 receptor antagonist abrogates the stimulation of osteoblast recruitment from bone marrow stromal cells by prostaglandin E2 in vivo and in vitro. *Bone* 34, 157-162.

Shen,F.W., Tung,J.S., and Boyse,E.A. (1986). Further definition of the Ly-5 system. *Immunogenetics* 24, 146-149.

Shi,S. and Klotz,U. (2008). Clinical use and pharmacological properties of selective COX-2 inhibitors. *Eur. J. Clin. Pharmacol.* 64, 233-252.

Shizuru,J.A., Negrin,R.S., and Weissman,I.L. (2005). Hematopoietic stem and progenitor cells: clinical and preclinical regeneration of the hematolymphoid system. *Annu. Rev. Med.* 56, 509-538.

Siegal,T. and Pfeffer,M.R. (1995). Radiation-induced changes in the profile of spinal cord serotonin, prostaglandin synthesis, and vascular permeability. *Int J Radiat. Oncol Biol Phys.* 31, 57-64.

Sigal,E. (1991). The molecular biology of mammalian arachidonic acid metabolism. *Am. J. Physiol* 260, L13-L28.

Siminovitch,L., McCulloch,E.A., and Till,J.E. (1963). The Distribution of Colony-forming Cells Among Spleen Colonies. *J. Cell Physiol* 62, 327-336.

Singh,H. (1996). Gene targeting reveals a hierarchy of transcription factors regulating specification of lymphoid cell fates. *Curr. Opin. Immunol.* 8, 160-165.

Singh, P., Hoggatt, J., Speth, J. M., and Pelus, L. M. Cyclooxygenase-2 Derived Prostaglandin E2 Is Required for Dendritic Cell Differentiation From Hematopoietic Progenitor Cells. *ASH Annual Meeting Abstracts* 114, 1499. 11-20-2009.

Sipkins,D.A., Wei,X., Wu,J.W., Runnels,J.M., Cote,D., Means,T.K., Luster,A.D., Scadden,D.T., and Lin,C.P. (2005). In vivo imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature* 435, 969-973.

Smith,G.C., Coleman,R.A., and McGrath,J.C. (1994). Characterization of dilator prostanoid receptors in the fetal rabbit ductus arteriosus. *J Pharmacol Exp Ther* 271, 390-396.

Smith,S. and Broxmeyer,H.E. (1986). The influence of oxygen tension on the long-term growth in vitro of haematopoietic progenitor cells from human cord blood. *Br. J Haematol.* 63, 29-34.

Smith,W.L., Marnett,L.J., and DeWitt,D.L. (1991). Prostaglandin and thromboxane biosynthesis. *Pharmacol. Ther.* 49, 153-179.

Souza,L.M., Boone,T.C., Gabrilove,J., Lai,P.H., Zsebo,K.M., Murdock,D.C., Chazin,V.R., Bruszewski,J., Lu,H., and Chen,K.K. (1986). Recombinant human granulocyte colony-stimulating factor: effects on normal and leukemic myeloid cells. *Science* 232, 61-65.

Spangrude,G.J., Heimfeld,S., and Weissman,I.L. (1988). Purification and characterization of mouse hematopoietic stem cells. *Science* 241, 58-62.

Srour,E.F., Brandt,J.E., Briddell,R.A., Leemhuis,T., van,B.K., and Hoffman,R. (1991). Human CD34+ HLA-DR- bone marrow cells contain progenitor cells capable of self-renewal, multilineage differentiation, and long-term in vitro hematopoiesis. *Blood Cells* 17, 287-295.

Srour,E.F., Zanjani,E.D., Cornetta,K., Traycoff,C.M., Flake,A.W., Hedrick,M., Brandt,J.E., Leemhuis,T., and Hoffman,R. (1993). Persistence of human multilineage, self-renewing lymphohematopoietic stem cells in chimeric sheep. *Blood* 82, 3333-3342.

Staller,P., Sulitkova,J., Lisztwan,J., Moch,H., Oakeley,E.J., and Krek,W. (2003). Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature* 425, 307-311.

Stem Cell Trialists' Group (2005). Allogeneic peripheral blood stem-cell compared with bone marrow transplantation in the management of hematologic malignancies: an individual patient data meta-analysis of nine randomized trials. *J. Clin. Oncol.* 23, 5074-5087.

Stengel,P.W., Cockerham,S.L., and Silbaugh,S.A. (2007). Inhaled anandamide reduces leukotriene D4-induced airway obstruction in guinea pigs. *Eur. J. Pharmacol.* 557, 66-68.

Stenke,L., Mansour,M., Edenius,C., Reizenstein,P., and Lindgren,J.A. (1991). Formation and proliferative effects of lipoxins in human bone marrow. *Biochem. Biophys. Res. Commun.* 180, 255-261.

Stenke,L., Mansour,M., Reizenstein,P., and Lindgren,J.A. (1993). Stimulation of human myelopoiesis by leukotrienes B4 and C4: interactions with granulocyte-macrophage colony-stimulating factor. *Blood* 81, 352-356.

- Stephenson,J.R., Axelrad,A.A., McLeod,D.L., and Shreeve,M.M. (1971). Induction of colonies of hemoglobin-synthesizing cells by erythropoietin in vitro. *Proc. Natl. Acad. Sci. U. S. A* 68, 1542-1546.
- Stier,S., Cheng,T., Dombkowski,D., Carlesso,N., and Scadden,D.T. (2002). Notch1 activation increases hematopoietic stem cell self-renewal in vivo and favors lymphoid over myeloid lineage outcome. *Blood* 99, 2369-2378.
- Stiff,P., Gingrich,R., Luger,S., Wyres,M.R., Brown,R.A., LeMaistre,C.F., Perry,J., Schenkein,D.P., List,A., Mason,J.R., Bensinger,W., Wheeler,C., Freter,C., Parker,W.R.L., and Emmanouilides,C. (2000). A randomized phase 2 study of PBPC mobilization by stem cell factor and filgrastim in heavily pretreated patients with Hodgkin's disease or non-Hodgkin's lymphoma. *Bone Marrow Transplant.* 26, 471-481.
- Strand,V. (2007). Are COX-2 inhibitors preferable to non-selective non-steroidal anti-inflammatory drugs in patients with risk of cardiovascular events taking low-dose aspirin? *Lancet* 370, 2138-2151.
- Strauer,B.E., Brehm,M., Zeus,T., Kostering,M., Hernandez,A., Sorg,R.V., Kogler,G., and Wernet,P. (2002). Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation* 106, 1913-1918.
- Stroncek,D., Shawker,T., Follmann,D., and Leitman,S.F. (2003). G-CSF-induced spleen size changes in peripheral blood progenitor cell donors. *Transfusion* 43, 609-613.
- Sugimoto,Y. and Narumiya,S. (2007). Prostaglandin E receptors. *J. Biol. Chem.* 282, 11613-11617.
- Sugiyama,T., Kohara,H., Noda,M., and Nagasawa,T. (2006). Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity.* 25, 977-988.
- Sutherland,D.R., Yeo,E.L., Stewart,A.K., Nayar,R., DiGiusto,R., Zanjani,E., Hoffman,R., and Murray,L.J. (1996). Identification of CD34+ subsets after glycoprotease selection: engraftment of CD34+Thy-1+Lin- stem cells in fetal sheep. *Exp. Hematol.* 24, 795-806.
- Sutherland,H.J., Eaves,C.J., Eaves,A.C., Dragowska,W., and Lansdorp,P.M. (1989). Characterization and partial purification of human marrow cells capable of initiating long-term hematopoiesis in vitro. *Blood* 74, 1563-1570.

- Sutherland,H.J., Lansdorp,P.M., Henkelman,D.H., Eaves,A.C., and Eaves,C.J. (1990). Functional characterization of individual human hematopoietic stem cells cultured at limiting dilution on supportive marrow stromal layers. *Proc Natl Acad Sci U. S. A* 87, 3584-3588.
- Suzawa,T., Miyaura,C., Inada,M., Maruyama,T., Sugimoto,Y., Ushikubi,F., Ichikawa,A., Narumiya,S., and Suda,T. (2000). The role of prostaglandin E receptor subtypes (EP1, EP2, EP3, and EP4) in bone resorption: an analysis using specific agonists for the respective EPs. *Endocrinology* 141, 1554-1559.
- Sweeney,E.A., Lortat-Jacob,H., Priestley,G.V., Nakamoto,B., and Papayannopoulou,T. (2002). Sulfated polysaccharides increase plasma levels of SDF-1 in monkeys and mice: involvement in mobilization of stem/progenitor cells. *Blood* 99, 44-51.
- Szczeklik,A. and Sanak,M. (2006). The broken balance in aspirin hypersensitivity. *Eur. J. Pharmacol.* 533, 145-155.
- Szilvassy,S.J., Lansdorp,P.M., Humphries,R.K., Eaves,A.C., and Eaves,C.J. (1989). Isolation in a single step of a highly enriched murine hematopoietic stem cell population with competitive long-term repopulating ability. *Blood* 74, 930-939.
- Szilvassy,S.J., Humphries,R.K., Lansdorp,P.M., Eaves,A.C., and Eaves,C.J. (1990). Quantitative assay for totipotent reconstituting hematopoietic stem cells by a competitive repopulation strategy. *Proc. Natl. Acad. Sci. U. S. A* 87, 8736-8740.
- Tabatabai,G., Frank,B., Mohle,R., Weller,M., and Wick,W. (2006). Irradiation and hypoxia promote homing of haematopoietic progenitor cells towards gliomas by TGF-beta-dependent HIF-1alpha-mediated induction of CXCL12. *Brain* 129, 2426-2435.
- Taetle,R. and Koessler,A. (1980). Effects of cyclic nucleotides and prostaglandins on normal and abnormal human myeloid progenitor proliferation. *Cancer Res.* 40, 1223-1229.
- Taetle,R., Guittard,J.P., and Mendelsohn,J.M. (1980). Abnormal modulation of granulocyte/macrophage progenitor proliferation by prostaglandin E in chronic myeloproliferative disorders. *Exp. Hematol.* 8, 1190-1201.
- Taichman,R.S. and Emerson,S.G. (1994). Human osteoblasts support hematopoiesis through the production of granulocyte colony-stimulating factor. *J Exp Med* 179, 1677-1682.

Taichman,R.S., Reilly,M.J., and Emerson,S.G. (2000). The Hematopoietic Microenvironment: Osteoblasts and The Hematopoietic Microenvironment. *Hematology*. 4, 421-426.

Takahashi,T., Kalka,C., Masuda,H., Chen,D., Silver,M., Kearney,M., Magner,M., Isner,J.M., and Asahara,T. (1999). Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. *Nat. Med* 5, 434-438.

Takeuchi,K., Kato,S., Takeeda,M., Ogawa,Y., Nakashima,M., and Matsumoto,M. (2003). Facilitation by endogenous prostaglandins of capsaicin-induced gastric protection in rodents through EP2 and IP receptors. *J. Pharmacol. Exp. Ther.* 304, 1055-1062.

Tamm,I., Wang,Y., Sausville,E., Scudiero,D.A., Vigna,N., Oltersdorf,T., and Reed,J.C. (1998). IAP-family protein survivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs. *Cancer Res.* 58, 5315-5320.

Taswell,C. (1981). Limiting dilution assays for the determination of immunocompetent cell frequencies. I. Data analysis. *J. Immunol.* 126, 1614-1619.

Taylor,K.M., Jagannath,S., Spitzer,G., Spinolo,J.A., Tucker,S.L., Fogel,B., Cabanillas,F.F., Hagemeister,F.B., and Souza,L.M. (1989). Recombinant human granulocyte colony-stimulating factor hastens granulocyte recovery after high-dose chemotherapy and autologous bone marrow transplantation in Hodgkin's disease. *J. Clin. Oncol.* 7, 1791-1799.

Terstappen,L.W., Huang,S., Safford,M., Lansdorp,P.M., and Loken,M.R. (1991). Sequential generations of hematopoietic colonies derived from single nonlineage-committed CD34+. *Blood* 77, 1218-1227.

Tesio,M., Gammaitoni,L., Gunetti,M., Leuci,V., Pignochino,Y., Jordaney,N., Capellero,S., Cammarata,C., Caione,L., Migliaretti,G., Fagioli,F., Tabilio,A., Aglietta,M., and Piacibello,W. (2008). Sustained long-term engraftment and transgene expression of peripheral blood CD34+ cells transduced with third-generation lentiviral vectors. *Stem Cells* 26, 1620-1627.

Thomas,B.F., Gilliam,A.F., Burch,D.F., Roche,M.J., and Seltzman,H.H. (1998). Comparative receptor binding analyses of cannabinoid agonists and antagonists. *J Pharmacol Exp Ther* 285, 285-292.

- Thompson,C., Cloutier,A., Bosse,Y., Poisson,C., Larivee,P., McDonald,P.P., Stankova,J., and Rola-Pleszczynski,M. (2008). Signaling by the cysteinyl-leukotriene receptor 2. Involvement in chemokine gene transcription. *J. Biol. Chem.* 283, 1974-1984.
- Till,J.E. and McCulloch,E.A. (1961). A direct measurement of the radiation sensitivity of normal mouse bone marrow cells. *Radiat. Res.* 14, 213-222.
- Till,J.E. and McCulloch,E.A. (1964). Repair Processes in Irradiated Mouse Hematopoietic Tissue. *Ann. N. Y. Acad. Sci.* 114, 115-125.
- To,L.B., Haylock,D.N., Simmons,P.J., and Juttner,C.A. (1997). The biology and clinical uses of blood stem cells. *Blood* 89, 2233-2258.
- Tomita,M., Li,X., Okada,Y., Woodiel,F.N., Young,R.N., Pilbeam,C.C., and Raisz,L.G. (2002). Effects of selective prostaglandin EP4 receptor antagonist on osteoclast formation and bone resorption in vitro. *Bone* 30, 159-163.
- Traycoff,C.M., Abboud,M.R., Laver,J., Brandt,J.E., Hoffman,R., Law,P., Ishizawa,L., and Srour,E.F. (1994). Evaluation of the in vitro behavior of phenotypically defined populations of umbilical cord blood hematopoietic progenitor cells. *Exp. Hematol.* 22, 215-222.
- Trentin,J.J. (1956). Mortality and skin transplantability in x-irradiated mice receiving isologous, homologous or heterologous bone marrow. *Proc. Soc. Exp. Biol. Med.* 92, 688-693.
- Tsuboi,K., Sugimoto,Y., and Ichikawa,A. (2002). Prostanoid receptor subtypes. *Prostaglandins Other Lipid Mediat.* 68-69, 535-556.
- Ueda,N. and Yamamoto,S. (2000). Anandamide amidohydrolase (fatty acid amide hydrolase). *Prostaglandins Other Lipid Mediat.* 61, 19-28.
- Ulbrich,H., Soehnlein,O., Xie,X., Eriksson,E.E., Lindbom,L., Albrecht,W., Laufer,S., and Dannhardt,G. (2005). Licofelone, a novel 5-LOX/COX-inhibitor, attenuates leukocyte rolling and adhesion on endothelium under flow. *Biochem. Pharmacol.* 70, 30-36.
- Uozumi,N., Kume,K., Nagase,T., Nakatani,N., Ishii,S., Tashiro,F., Komagata,Y., Maki,K., Ikuta,K., Ouchi,Y., Miyazaki,J., and Shimizu,T. (1997). Role of cytosolic phospholipase A2 in allergic response and parturition. *Nature* 390, 618-622.
- Uozumi,N. and Shimizu,T. (2002). Roles for cytosolic phospholipase A2alpha as revealed by gene-targeted mice. *Prostaglandins Other Lipid Mediat.* 68-69, 59-69.

- Urade,Y., Watanabe,K., and Hayaishi,O. (1995). Prostaglandin D, E, and F synthases. *J. Lipid Mediat. Cell Signal.* 12, 257-273.
- Vadhan-Raj,S., Al Katib,A., Bhalla,R., Pelus,L., Nathan,C.F., Sherwin,S.A., Oettgen,H.F., and Krown,S.E. (1986). Phase I trial of recombinant interferon gamma in cancer patients. *J Clin Oncol* 4, 137-146.
- Valk,P.J., Verbakel,S., Vankan,Y., Hol,S., Mancham,S., Ploemacher,R., Mayen,A., Lowenberg,B., and Delwel,R. (1997). Anandamide, a natural ligand for the peripheral cannabinoid receptor is a novel synergistic growth factor for hematopoietic cells. *Blood* 90, 1448-1457.
- Valk,P.J. and Delwel,R. (1998). The peripheral cannabinoid receptor, Cb2, in retrovirally-induced leukemic transformation and normal hematopoiesis. *Leuk. Lymphoma* 32, 29-43.
- van der Loo,J.C., Xiao,X., McMillin,D., Hashino,K., Kato,I., and Williams,D.A. (1998). VLA-5 is expressed by mouse and human long-term repopulating hematopoietic cells and mediates adhesion to extracellular matrix protein fibronectin. *J. Clin. Invest* 102, 1051-1061.
- van Hennik,P.B., de Koning,A.E., and Ploemacher,R.E. (1999). Seeding efficiency of primitive human hematopoietic cells in nonobese diabetic/severe combined immune deficiency mice: implications for stem cell frequency assessment. *Blood* 94, 3055-3061.
- Varnum-Finney,B., Brashem-Stein,C., and Bernstein,I.D. (2003). Combined effects of Notch signaling and cytokines induce a multiple log increase in precursors with lymphoid and myeloid reconstituting ability. *Blood* 101, 1784-1789.
- Velders,G.A., Pruijt,J.F., Verzaal,P., van,O.R., van,K.Y., Figdor,C.G., de Kruijf,E.J., Willemze,R., and Fibbe,W.E. (2002). Enhancement of G-CSF-induced stem cell mobilization by antibodies against the beta 2 integrins LFA-1 and Mac-1. *Blood* 100, 327-333.
- Verma,D.S., Spitzer,G., Zander,A.R., McCredie,K.B., and Dicke,K.A. (1981). Prostaglandin E1-mediated augmentation of human granulocyte-macrophage progenitor cell growth in vitro. *Leuk. Res.* 5, 65-71.
- Vermeulen,M., Le,P.F., Gagnerault,M.C., Mary,J.Y., Sainteny,F., and Lepault,F. (1998). Role of adhesion molecules in the homing and mobilization of murine hematopoietic stem and progenitor cells. *Blood* 92, 894-900.

Villablanca,E.J., Pistocchi,A., Court FA, Cotelli,F., Bordignon,C., Allende,M.L., Traversari,C., and Russo,V. (2007). Abrogation of prostaglandin E2/EP4 signaling impairs the development of rag1+ lymphoid precursors in the thymus of zebrafish embryos. *J. Immunol.* 179, 357-364.

Visnjic,D., Kalajzic,Z., Rowe,D.W., Katavic,V., Lorenzo,J., and Aguila,H.L. (2004). Hematopoiesis is severely altered in mice with an induced osteoblast deficiency. *Blood* 103, 3258-3264.

Vittorio,P.V. and Whitfield,J.F. (1971). The beneficial effects of postirradiation treatment with cobaltous chloride on the development of nuclear damage in thymocytes, erythropoiesis, and the survival of mice. *Can. J. Physiol Pharmacol.* 49, 812-818.

Volpi,I., Perruccio,K., Tosti,A., Capanni,M., Ruggeri,L., Posati,S., Aversa,F., Tabilio,A., Romani,L., Martelli,M.F., and Velardi,A. (2001). Postgrafting administration of granulocyte colony-stimulating factor impairs functional immune recovery in recipients of human leukocyte antigen haplotype-mismatched hematopoietic transplants. *Blood* 97, 2514-2521.

Von Euler,U.S. (1936). On the specific vaso-dilating and plain muscle stimulating substances from accessory genital glands in man and certain animals (prostaglandin and vesiglandin). *J. Physiol* 88, 213-234.

Vore,S.J., Eling,T.E., Danilowicz,M., Tucker,A.N., and Luster,M.I. (1989). Regulation of murine hematopoiesis by arachidonic acid metabolites. *Int. J. Immunopharmacol.* 11, 435-442.

Vos,O. and Dolmans,M.J. (1972). Self-renewal of colony forming units (CFU) in serial bone marrow transplantation experiments. *Cell Tissue Kinet.* 5, 371-385.

Vose,J.M., Bierman,P.J., Lynch,J.C., Atkinson,K., Juttner,C., Hanania,C.E., Bociek,G., and Armitage,J.O. (2001). Transplantation of highly purified CD34+Thy-1+ hematopoietic stem cells in patients with recurrent indolent non-Hodgkin's lymphoma. *Biol. Blood Marrow Transplant.* 7, 680-687.

Wald,N. (1982). Radiation Injury. In Cecil Textbook of Medicine, J.B.Wyngaarden and L.H.J.Smith, eds. (Philadelphia: W.B. Saunders), p. 2228.

Walden,T.L., Jr., Patchen,M., and Snyder,S.L. (1987). 16,16-Dimethyl prostaglandin E2 increases survival in mice following irradiation. *Radiat. Res.* 109, 440-448.

Walden,T.L., Jr. and Farzaneh,N.K. (1995). Radioprotection by 16,16 dimethyl prostaglandin E2 is equally effective in male and female mice. *J. Radiat. Res. (Tokyo)* 36, 1-7.

- Wan,X.S., Ware,J.H., Zhou,Z., Donahue,J.J., Guan,J., and Kennedy,A.R. (2006). Protection against radiation-induced oxidative stress in cultured human epithelial cells by treatment with antioxidant agents. *Int. J. Radiat. Oncol. Biol. Phys.* **64**, 1475-1481.
- Wang,D., Mann,J.R., and DuBois,R.N. (2004). WNT and cyclooxygenase-2 cross-talk accelerates adenoma growth. *Cell Cycle* **3**, 1512-1515.
- Wang,G.L. and Semenza,G.L. (1993). General involvement of hypoxia-inducible factor 1 in transcriptional response to hypoxia. *Proc. Natl. Acad. Sci. U. S. A* **90**, 4304-4308.
- Wang,L.S., Liu,H.J., Xia,Z.B., Broxmeyer,H.E., and Lu,L. (2000). Expression and activation of caspase-3/CPP32 in CD34(+) cord blood cells is linked to apoptosis after growth factor withdrawal. *Exp. Hematol.* **28**, 907-915.
- Watt,S.M., Karhi,K., Gatter,K., Furley,A.J., Katz,F.E., Healy,L.E., Altass,L.J., Bradley,N.J., Sutherland,D.R., Levinsky,R., and . (1987). Distribution and epitope analysis of the cell membrane glycoprotein (HPCA-1) associated with human hemopoietic progenitor cells. *Leukemia* **1**, 417-426.
- Weber,A., Ni,J., Ling,K.H., Acheampong,A., Tang-Liu,D.D., Burk,R., Cravatt,B.F., and Woodward,D. (2004). Formation of prostamides from anandamide in FAAH knockout mice analyzed by HPLC with tandem mass spectrometry. *J. Lipid Res.* **45**, 757-763.
- Wei,H., Wang,C., and Chen,L. (2006). Proliferating cell nuclear antigen, survivin, and CD34 expressions in pancreatic cancer and their correlation with hypoxia-inducible factor 1alpha. *Pancreas* **32**, 159-163.
- Weinreb,M., Shamir,D., Machwate,M., Rodan,G.A., Harada,S., and Keila,S. (2006). Prostaglandin E2 (PGE2) increases the number of rat bone marrow osteogenic stromal cells (BMSC) via binding the EP4 receptor, activating sphingosine kinase and inhibiting caspase activity. *Prostaglandins Leukot. Essent. Fatty Acids* **75**, 81-90.
- Weissman,I.L. and Shizuru,J.A. (2008). The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. *Blood* **112**, 3543-3553.
- Welte,K., Bonilla,M.A., Gillio,A.P., Boone,T.C., Potter,G.K., Gabrilove,J.L., Moore,M.A., O'Reilly,R.J., and Souza,L.M. (1987). Recombinant human granulocyte colony-stimulating factor. Effects on hematopoiesis in normal and cyclophosphamide-treated primates. *J. Exp. Med.* **165**, 941-948.

Wen,Q., Leung,C., Huang,Z., Small,S., Reddi,A.L., Licht,J.D., and Crispino,J.D. (2009). Survivin is not required for the endomitotic cell cycle of megakaryocytes. *Blood* 114, 153-156.

Whitlock,C.A. and Witte,O.N. (1982). Long-term culture of B lymphocytes and their precursors from murine bone marrow. *Proc. Natl. Acad. Sci. U. S. A* 79, 3608-3612.

Whitlock,C.A., Robertson,D., and Witte,O.N. (1984). Murine B cell lymphopoiesis in long term culture. *J. Immunol. Methods* 67, 353-369.

Willeit,C.G., Czito,B.G., Bendell,J.C., and Ryan,D.P. (2005). Locally advanced pancreatic cancer. *J. Clin. Oncol.* 23, 4538-4544.

Wilson,A., Laurenti,E., Oser,G., van der Wath,R.C., Blanco-Bose,W., Jaworski,M., Offner,S., Dunant,C.F., Eshkind,L., Bockamp,E., Lio,P., MacDonald,H.R., and Trumpp,A. (2008). Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. *Cell* 135, 1118-1129.

Wolfram,O., Jentsch-Ullrich,K., Wagner,A., Hammwohner,M., Steinke,R., Franke,A., Zupan,I., Klein,H.U., and Goette,A. (2007). G-CSF-induced mobilization of CD34(+) progenitor cells and proarrhythmic effects in patients with severe coronary artery disease. *Pacing Clin. Electrophysiol.* 30 Suppl 1, S166-S169.

Wong,D.A., Kita,Y., Uozumi,N., and Shimizu,T. (2002). Discrete role for cytosolic phospholipase A(2)alpha in platelets: studies using single and double mutant mice of cytosolic and group IIA secretory phospholipase A(2). *J. Exp. Med.* 196, 349-357.

Wright,D.E., Wagers,A.J., Gulati,A.P., Johnson,F.L., and Weissman,I.L. (2001). Physiological migration of hematopoietic stem and progenitor cells. *Science* 294, 1933-1936.

Wu,A.M., Till,J.E., Siminovitch,L., and McCulloch,E.A. (1967). A cytological study of the capacity for differentiation of normal hemopoietic colony-forming cells. *J. Cell Physiol* 69, 177-184.

Wu,A.M., Till,J.E., Siminovitch,L., and McCulloch,E.A. (1968). Cytological evidence for a relationship between normal hemotopoietic colony-forming cells and cells of the lymphoid system. *J. Exp. Med.* 127, 455-464.

Wu,M., Kwon,H.Y., Rattis,F., Blum,J., Zhao,C., Ashkenazi,R., Jackson,T.L., Gaiano,N., Oliver,T., and Reya,T. (2007). Imaging hematopoietic precursor division in real time. *Cell Stem Cell* 1, 541-554.

- Xie,Y., Yin,T., Wiegraebe,W., He,X.C., Miller,D., Stark,D., Perko,K., Alexander,R., Schwartz,J., Grindley,J.C., Park,J., Haug,J.S., Wunderlich,J.P., Li,H., Zhang,S., Johnson,T., Feldman,R.A., and Li,L. (2009). Detection of functional haematopoietic stem cell niche using real-time imaging. *Nature* **457**, 97-101.
- Yang,L., Cao,Z., Li,F., Post,D.E., Van Meir,E.G., Zhong,H., and Wood,W.C. (2004). Tumor-specific gene expression using the survivin promoter is further increased by hypoxia. *Gene Ther.* **11**, 1215-1223.
- Yang,L., Bryder,D., Adolfsson,J., Nygren,J., Mansson,R., Sigvardsson,M., and Jacobsen,S.E. (2005). Identification of Lin(-)Sca1(+)kit(+)CD34(+)Flt3- short-term hematopoietic stem cells capable of rapidly reconstituting and rescuing myeloablated transplant recipients. *Blood* **105**, 2717-2723.
- Yao,L., Yokota,T., Xia,L., Kincade,P.W., and McEver,R.P. (2005). Bone marrow dysfunction in mice lacking the cytokine receptor gp130 in endothelial cells. *Blood* **106**, 4093-4101.
- Yilmaz,O.H., Kiel,M.J., and Morrison,S.J. (2006). SLAM family markers are conserved among hematopoietic stem cells from old and reconstituted mice and markedly increase their purity. *Blood* **107**, 924-930.
- Yin,A.H., Miraglia,S., Zanjani,E.D., Almeida-Porada,G., Ogawa,M., Leary,A.G., Olweus,J., Kearney,J., and Buck,D.W. (1997). AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* **90**, 5002-5012.
- Yoder,M.C. and Williams,D.A. (1995). Matrix molecule interactions with hematopoietic stem cells. *Exp. Hematol.* **23**, 961-967.
- Yoder,M.C., Mead,L.E., Prater,D., Krier,T.R., Mroueh,K.N., Li,F., Krasich,R., Temm,C.J., Prchal,J.T., and Ingram,D.A. (2007). Redefining endothelial progenitor cells via clonal analysis and hematopoietic stem/progenitor cell principals. *Blood* **109**, 1801-1809.
- Yoder,M.C. (2009). Defining human endothelial progenitor cells. *J. Thromb. Haemost.* **7 Suppl 1**, 49-52.
- Yokomizo,T., Izumi,T., and Shimizu,T. (2001). Leukotriene B4: metabolism and signal transduction. *Arch. Biochem. Biophys.* **385**, 231-241.
- Yoshida,K., Oida,H., Kobayashi,T., Maruyama,T., Tanaka,M., Katayama,T., Yamaguchi,K., Segi,E., Tsuboyama,T., Matsushita,M., Ito,K., Ito,Y., Sugimoto,Y., Ushikubi,F., Ohuchida,S., Kondo,K., Nakamura,T., and Narumiya,S. (2002). Stimulation of bone formation and prevention of bone loss by prostaglandin E EP4 receptor activation. *Proc Natl Acad Sci U. S. A* **99**, 4580-4585.

- Yu,M., Ives,D., and Ramesha,C.S. (1997). Synthesis of prostaglandin E2 ethanolamide from anandamide by cyclooxygenase-2. *J. Biol. Chem.* 272, 21181-21186.
- Yuan,Y., Hilliard,G., Ferguson,T., and Millhorn,D.E. (2003). Cobalt inhibits the interaction between hypoxia-inducible factor-alpha and von Hippel-Lindau protein by direct binding to hypoxia-inducible factor-alpha. *J. Biol. Chem.* 278, 15911-15916.
- Zanjani,E.D., Pallavicini,M.G., Ascensao,J.L., Flake,A.W., Langlois,R.G., Reitsma,M., MacKintosh,F.R., Stutes,D., Harrison,M.R., and Tavassoli,M. (1992). Engraftment and long-term expression of human fetal hemopoietic stem cells in sheep following transplantation in utero. *J. Clin. Invest* 89, 1178-1188.
- Zanjani,E.D., Almeida-Porada,G., Livingston,A.G., Flake,A.W., and Ogawa,M. (1998). Human bone marrow CD34- cells engraft in Vivo and undergo multilineage expression that includes giving rise to CD34+ cells. *Exp. Hematol.* 26, 353-360.
- Zanjani,E.D., Flake,A.W., Almeida-Porada,G., Tran,N., and Papayannopoulou,T. (1999). Homing of human cells in the fetal sheep model: modulation by antibodies activating or inhibiting very late activation antigen-4-dependent function. *Blood* 94, 2515-2522.
- Zhai,Q.L., Qiu,L.G., Li,Q., Meng,H.X., Han,J.L., Herzig,R.H., and Han,Z.C. (2004). Short-term ex vivo expansion sustains the homing-related properties of umbilical cord blood hematopoietic stem and progenitor cells. *Haematologica* 89, 265-273.
- Zhang,J., Niu,C., Ye,L., Huang,H., He,X., Tong,W.G., Ross,J., Haug,J., Johnson,T., Feng,J.Q., Harris,S., Wiedemann,L.M., Mishina,Y., and Li,L. (2003). Identification of the haematopoietic stem cell niche and control of the niche size. *Nature* 425, 836-841.
- Zhao,W., Diz,D.I., and Robbins,M.E. (2007). Oxidative damage pathways in relation to normal tissue injury. *Br. J. Radiol.* 80 *Spec No 1*, S23-S31.
- Zhou,L., Pope,B.L., Chourmouzis,E., Fung-Leung,W.P., and Lau,C.Y. (1996). Tepoxalin blocks neutrophil migration into cutaneous inflammatory sites by inhibiting Mac-1 and E-selectin expression. *Eur. J. Immunol.* 26, 120-129.
- Zohren,F., Toutzaris,D., Klarner,V., Hartung,H.P., Kieseier,B., and Haas,R. (2008). The monoclonal anti-VLA-4 antibody natalizumab mobilizes CD34+ hematopoietic progenitor cells in humans. *Blood* 111, 3893-3895.

Zou, Y.R., Kottmann, A.H., Kuroda, M., Taniuchi, I., and Littman, D.R. (1998).
Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar
development. *Nature* 393, 595-599.

Curriculum Vitae

Jonathan G. Hoggatt

Education

| | | |
|------|---|--------------------|
| 2005 | B.S. Pharmaceutical Sciences Minor in Psychology Purdue University | West Lafayette, IN |
| 2006 | M.S. Biological Sciences IUPUI | Indianapolis, IN |
| 2010 | Ph.D. Department of Microbiology & Immunology, Indiana University | Indianapolis, IN |

Activities and Honors

| | |
|--------------|--|
| 2007-2009 | Police Merit Commissioner for West Lafayette, Indiana |
| 2009-present | City Councilor for West Lafayette, Indiana |
| 2001 | Eli Lilly Pharmacy Scholarship |
| 2001-2002 | Health Sciences Freshman Scholar – Given to only 15 incoming freshman to support undergraduate research |
| 2002-2004 | Angelo J. Carnaghi Pharmacy Scholarship |
| 2002 | EMV Outstanding Leader Award – One of only 4 sophomore leaders chosen as the most outstanding at Purdue University |
| 2003 | Betty M. Nelson Skilled Leader Award – Given to the most outstanding Junior leader at Purdue University |
| 2003 | Varsity Letter Winner in Cross Country |
| 2003-2005 | Varsity Letter Winner in Track and Field |
| 2003-2005 | Academic All Big Ten in Track and Field |
| 2004 | Academic All Big Ten in Cross Country |
| 2005 | Merck Award Winner |
| 2006-2010 | Graduate Fellow on NIH Training Grant T32 DK07519 “Regulation of Hematopoietic Cells Production” |

Work Experience

| | |
|------------|---|
| 2003, 2004 | US Army Breast Cancer Fellowship <ul style="list-style-type: none">• Recipient of two separate summer fellowships at Purdue University, West Lafayette, Indiana• Developed mass spectrometric techniques for protein post-translational modification detection |
|------------|---|

Work Experience

- 2005 Pharmaceutical Intern; Hospira Inc.; Lake Forest, Illinois
- Created standard operating procedures for generic parenteral research and development methods.
 - Determined impurity and potency levels for pharmaceutical products utilizing developed methods.
- 2005-2006 English as a Second Language Tutor
- Tutored international students, ranging in age from 10 to 40 years old, on an individual basis to develop and improve English proficiency
 - Created unique lesson plans and assignments
- 2006 Pharmaceutical Intern; Hospira Inc.; Lake Forest, Illinois
- Developed a novel moisture determination procedure allowing for in-line analysis of lyophilized products
 - Developed a manufacturing formulation protocol for a proprietary parenteral product
 - Created standard operating procedure for determination of ion impurities via Ion Chromatography

Publications

Hoggatt, J.; Singh, P.; Sampath, J. and Pelus, L.M. (2009). Prostaglandin E₂ enhances hematopoietic stem cell homing, survival, and proliferation. *Blood*. May 28;113(22):5444-55.

Pelus, L.M.; **Hoggatt, J.;** Singh, P. and Sampath, J. (2009). Hematopoietic Agents. *Burger's Medicinal Chemistry*. (Accepted).

Hoggatt, A.; **Hoggatt, J.;** and Pelus, L.M. (2009). A spoonful of sugar helps the medicine go down: A novel technique to improve oral gavage in mice. *JAALAS*. (Accepted).

Singh, P.; **Hoggatt, J.;** Speth, J. and Pelus, L.M. (2010). Prostaglandin E₂ Regulates Dendritic Cell Differentiation from Bone Marrow Hematopoietic Progenitor Cells. *Blood*. (Submitted).

Abstracts

Hoggatt, J.; Singh, P.; Sampath, J. and Pelus, L.M. (2008). Prostaglandin E₂ enhances survival, proliferation, homing and engraftment of mouse hematopoietic stem cells. (Oral presentation at American Society of Hematology Conference).

Hoggatt, J.; Singh, P.; Hoggatt, A.; Speth, J. and Pelus, L.M., (2009). Inhibition of Prostaglandin E₂ (PGE₂) Signaling by Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) or EP4 Receptor Antagonism Expands Hematopoietic Stem and

Progenitor Cells (HSPC) and Enhances Their Mobilization to Peripheral Blood in Mice and Baboons. (Oral presentation at ASH Conference).

Hoggatt, J.; Singh, P.; Hoggatt, A.; Speth, J. and Pelus, L.M. (2010). Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) or EP4 Receptor Antagonism Enhances Hematopoietic Stem and Progenitor Cell (HSPC) Mobilization and Facilitates Faster Hematopoietic Recovery After Myeloablation. (Poster Presentation at the American Society of Clinical Oncology).

Broxmeyer, H.E.; **Hoggatt, J.**; Cooper, S.; Hangoc, G.; Pelus, L.M. and Campbell, T. (2009). CD26/Dipeptidylpeptidase IV Regulates Potency of Selected Hematopoietic Growth Factors through Truncation, and Recovery In Vivo After Cytotoxic Stress. (Poster at ASH Conference).

Singh, P.; **Hoggatt, J.**; Speth, J. and Pelus, L.M. (2009). Cyclooxygenase-2 Derived Prostaglandin E2 Is Required for Dendritic Cell Differentiation From Hematopoietic Progenitor Cells. (Poster at ASH Conference).

Sool Yeon Cho; Mingjiang Xu; Pratibha Singh; **Jonathan Hoggatt**; Louis M. Pelus; Ronald Hoffman; John Roboz, 2009, "Mass spectrometric study of the truncation of stromal cell-derived factor-1 (SDF-1) by proteolytic enzymes in patients with myeloproliferative diseases", (Accepted at the American Society of Mass Spectrometry Conference).

Patent Applications

PCT Patent Application No. PCT/US09/63654 "Materials and Methods to Enhance Hematopoietic Stem Cell Engraftment Procedures" (Filed 11/06/08)

U.S. Provisional Application No. 61/261,352 "Inhibition of Prostaglandin E2 (PGE2) Signaling by Non-Steroidal Anti-Inflammatory Drugs"